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(71) Applicant *(for all designated States except US)*: NATIONAL RESEARCH COUNCIL OF CANADA [CA/CA]; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: ZOU, Jitao [CA/CA]; 10-1910 Main Street, Saskatoon, Saskatchewan S7H 0L8 (CA). TAYLOR, David, C. [CA/CA]; 622 Wollaston Bay, Saskatoon, Saskatchewan S7J 4C3 (CA). KATAVIC, Vesna [CA/CA]; 301-1121C McKercher Drive, Saskatoon, Saskatchewan S7H 5B8 (CA). MacKENZIE, Samuel, L. [CA/CA]; 17 Cambridge Crescent, Saskatoon, Saskatchewan S7H 3P9 (CA). KELLER, Wilfred, A. [CA/CA]; 234 Emmeline Road, Saskatoon, Saskatchewan S7J 5B6 (CA).

(74) Agents: GALE, Edwin, J. et al.; Kirby, Eades, Gale, Baker, Box 3432, Station D, Ottawa, Ontario K1P 6N9 (CA).

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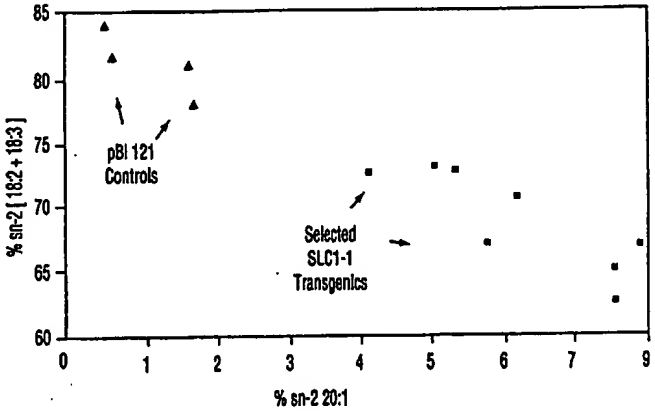

Correlation between decrease in *sn-2* polyunsaturated fatty acids and increase in *sn-2* eicosenoic acid in A. *thaliana* Control and *SLC1-1* Transgenic T_3 Seeds.

(57) Abstract

This invention relates to the modification of plant lipids and seed oils by genetic engineering techniques to produce oilseeds of enhanced commercial value. In one form, the invention relates to a transgenic oilseed plant, or a seed of such plant, having a genome incorporating an expressible yeast *SLC1-1* or *SLC1* gene. The invention also provides a method of producing a transgenic oilseed plant, which comprises introducing into the genome of the plant an expressible yeast *SLC1-1* or *SLC1* gene. The invention also relates to various plasmids and vectors used in the method of the invention.

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MODIFICATION OF PLANT LIPIDS AND SEED OILS UTILIZING YEAST SLC GENES

TECHNICAL FIELD

This invention relates to the modification of plant lipids and seed oils by genetic engineering techniques. More particularly, the invention relates to a method of genetically modifying oilseed plants to produce oilseeds or whole plants of enhanced commercial value. The invention also relates to the modified plants and seeds, and to genetic materials and vectors used for the production of such plants, and for further modifications of plants.

BACKGROUND ART

There is considerable interest nowadays in modifying the seed oil fatty acid composition and content of oilseeds by molecular genetic means to provide a dependable source of Super High Erucic Acid Rapeseed (SHEAR) oil for use as an industrial feedstock. A similar interest exists for producing other strategic non-edible oils (e.g. seed oils high in hydroxy-, epoxy-, short and medium chain fatty acids, etc.) in traditional oilseed crops (e.g. rapeseed, flax, sunflower, soybean).

For edible oils, there is considerable interest in changing the fatty acid composition (e.g. higher oleic/lower polyunsaturates, lower saturates, higher saturates) as well as increasing the oil content in oilseed crops such as Canola and edible oil flax (Linola), soybean and sunflower.

Currently, there are no documented demonstrations of increases in oil content (yield) by transgenic means, although yield increases by traditional breeding and selection continue to bring about incremental improvements.

In contrast, increases in the *proportions* of some strategic fatty acids have been achieved by the introduction of various plant fatty acid biosynthesis and acyltransferase genes in oilseeds. Some examples of such processes are the following:

1. Expression of a medium chain fatty acyl-ACP thioesterase from California Bay, in Brassicaceae to increase the lauric acid (12:0) content (Calgene; Voelker et al., 1995; 1996 - see References 35 and 36 in the accompanying "References Pertinent to the Present Invention").

2. Expression of a *Jojoba* β-ketoacyl-CoA synthase in low erucic acid *Brassica napus* (Canola) cultivars to increase the level of erucic acid; the effect following expression in high erucic acid cultivars was negligible (Calgene; Lassner et al., 1996 - see Reference 20).

3. Expression of an anti-sense construct to the stearoyl-ACP Δ9 desaturase in Brassicaceae to increase the stearic acid content (Calgene; Knutzon et al., 1992 - see Reference 16).

4. Increased proportions of oleic acid in *B. napus* by co-suppression using a sense construct encoding plant microsomal FAD2 (Δ12) desaturase (duPont/InterMountain Canola; Hitz et al., 1995 - see Reference 12).

5. Increased proportions of 12:0 or 22:1 in the *sn*-2 position of triacylglycerols (TAGs) in rapeseed by expression of coconut or meadowfoam *lyso*-phosphatidic acid acyltransferases (LPATs; E.C. 2.3.1.51), respectively (Calgene; Knutzon et al., 1995 a & b; - see References 17 and 18; Lassner et al., 1995 - see Reference 21).

Although the use of plant transgenes resulted in altered proportions of *sn*-2 lauric and erucic acids, in

laurate canola and high erucic acid rapeseed, respectively, the overall proportions of lauric and erucic acids in the seed oil were *not* increased, and there was no evidence of increased total fatty acid content, or increased oil yield in these transgenics.

There is accordingly a need for new ways of increasing oil yield and improving oil composition in oilseed plants by employing genetic engineering techniques.

DISCLOSURE OF INVENTION

An object of the present invention is to genetically modify oilseed plants to improve the commercial value of such plants, the seeds of such plants, and the oils produced from such plants.

Another object of the invention is to provide a method of modifying the yield and composition of oils derived from oilseed plants.

The present invention is based on the discovery that *sn*-2 acylglyceride fatty acyltransferase genes (*SLC1-1* and its allele, *SLC1*) from yeast (*Saccharomyces cereviseae*), can be used to change the oil content and oil composition of plant seed and leaf lipids.

Thus, according to one aspect of the present invention, there is provided a transgenic oilseed plant having a genome incorporating an expressible yeast *SLC1-1* or *SLC1* gene.

According to another aspect of the invention, there is provided a seed of a transgenic oilseed plant having a genome incorporating an expressible yeast *SLC1-1* or *SLC1* gene.

According to yet another aspect of the invention, there is provided a method of producing a transgenic oilseed plant, which comprises introducing into the genome of said plant an expressible yeast *SLC1-1* or *SLC1* gene.

The invention also relates to various plasmids and vectors used in the method of the invention, and to the co-

introduction of other genes into plants modified to include the *SLC1-1* and *SLC1* genes.

The advantages of the present invention include the fact that the yeast *SLC1-1* and *SLC1* genes can be used to increase the oil content and to change total fatty acid composition, as well as to alter the acyl composition of TAGs, including the *sn*-2 position, and to change the relative proportions of TAG species, in various oilseed plants, e.g. *Arabidopsis thaliana*, in high erucic acid and canola cultivars of *Brassica napus*, and in *Brassica carinata*.

Moreover, the yeast *sn*-2 acyltransferase (*SLC1-1* and *SLC1* genes) can be utilized in high erucic acid Brassicaceae to increase the oil content and to produce seed oils with increased content of very long-chain fatty acids (VLCFAs) and TAGs with an altered stereospecific composition with respect to very long chain fatty acids. Thus, in contrast to previous results utilizing plant transgenes (as mentioned above), the current invention utilizing a yeast transgene is capable of achieving combined increases in seed oil content, seed erucic acid content and overall proportions of erucic acid in the seed oil.

The yeast *sn*-2 acyltransferase (*SLC1-1* and *SLC1* genes) can also be utilized in edible oil cultivars (Canola-quality cultivars) of the Brassicaceae, to increase the oil content and to produce seed oils with altered proportions of oleic acid, polyunsaturated fatty acids and very long chain saturated fatty acids.

The related yeast *SLC1-1* and *SLC1* alleles can be utilized in the same ways. Both alleles encode an *sn*-2 acyltransferase; *SLC1* differs from *SLC1-1* only in the amino acid at position 44 (Glutamine, Q) compared to *SLC1-1*, where the amino acid at position 44 is Leucine (L).

The *SLC1-1* and *SLC1* transgenic plants can be used as host germplasm for further down-regulation of indigenous plant acyltransferases.

To achieve directed assembly of TAG biosynthesis to produce stereospecifically-designed TAGs, the co-ordinated expression of a number of biochemical reactions, including that mediated by LPAT, is required. One of the distinct possibilities with respect to optimizing transgenic expression of foreign LPATs to synthesize TAGs with new acyl compositions (e.g. increased very long chain fatty acids at the *sn*-2 position), is the possible need to simultaneously down-regulate the indigenous LPAT already present in the transgenic host (e.g. an LPAT which normally prefers to insert polyunsaturated C_{18} fatty acyl groups into the *sn*-2 position). The overall homologies between the yeast *sn*-2 acyltransferases and published plant *sn*-2 acyltransferases (LPATs) are low, and are restricted mostly to the C-termini of the proteins. In contrast, the plant acyltransferases have much greater overall homology to each other, and regions of homology extend throughout the sequence. Therefore, the use of the yeast *SLC* genes to achieve the effects described herein, allow a unique opportunity to further improve these traits in a way not possible when the initial transformation was performed with a plant acyltransferase. In effect, the limited homology between plant and the yeast *sn*-2 acyltransferases are low enough to allow strategies to down-regulate the host plant LPAT by conventional means (e.g. anti-sense RNA technology or a co-suppression phenomenon; Mol et al., 1990; Van Blokland et al., 1993; De Lange et al., 1995) without a concomitant negative impact on the expression of the yeast transgene or on plant seed development. Thus, the yeast transgene strategy has a distinct advantage over that in which another *plant* transgene is introduced into a host plant where there is a highly homologous, indigenous LPAT.

The yeast *sn*-2 acyltransferase (*SLC1-1* and *SLC1* genes) can be used to increase the oil content and alter the acyl composition of TAGs in all other oilseeds including borage (*Borago* spp.), castor (*Ricinus communis*), cocoa bean (*Theobroma cacao*), corn (*Zea mays*), cotton (*Gossypium* spp), *Crambe* spp., *Cuphea* spp., flax (*Linum* spp.), *Lesquerella* and *Limnanthes* spp., nasturtium (*Tropaeolum* spp.), *Oenothera* spp., olive (*Olea* spp.), palm (*Elaeis* spp.), peanut (*Arachis* spp.), safflower (*Carthamus* spp.), soybean (*Glycine* and *Soja* spp.), sunflower (*Helianthus* spp.), tobacco (*Nicotiana* spp.) and *Vernonia* spp.

The yeast *sn*-2 acyltransferase (*SLC1-1* and *SLC1* genes) oilseed transformants can be utilized, by a second transformation, with all other value-added fatty acid biosynthesis genes (e.g. the hydroxylase gene from castor or *Lesquerella* spp.), or by crossing with related oilseed transformants already containing such value-added genes, to produce seed oils with increased amounts of value-added fatty acids (e.g. increased hydroxy fatty acid content and altered TAG composition with respect to those containing hydroxy fatty acids).

The *SLC1-1* gene and related *SLC1 allele*, can be utilized to modify fatty acid and lipid profiles in vegetative tissues to improve tolerance to biotic and abiotic plant stresses (e.g. increased membrane fluidity in root and leaf tissues to improve frost tolerance).

The use of the yeast *SLC1-1* gene and the *SLC1* allele in plants, to bring about changes in overall lipid content and composition, has not been previously disclosed or demonstrated (reduced to practice) as a means for manipulating the relative proportions or amounts of fatty acids (e.g. very long chain fatty acids), and also for increasing the oil content of crops producing edible or industrial oils.

Previously, there have been no demonstrations of increases in oil yields brought about by transgenic means.

More specifically, there was no previous evidence that yeast acyltransferases, the enzymes responsible for synthesizing triacylglycerols, have been expressed in plants to alter oil composition or content.

In contrast, however, a decrease in diacylglycerol acyltransferase activity in a mutant of *Arabidopsis thaliana* resulted in a decrease in oil yield and a change in acyl composition (Katavic et al., (1995) *Plant Physiology*, 108:399-409 - see Reference 15).

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the nucleotide [SEQ ID NO:1] and deduced amino acid sequence [SEQ ID NO:2] of the coding region of the yeast *SLC1-1* gene used in the present invention, the stop codon being identified by "@", and a highly conserved consensus sequence among bacterial and yeast *sn*-2 acyltransferases being underlined;

Fig. 2 shows the nucleotide [SEQ ID NO:3] and deduced amino acid sequence [SEQ ID NO:4] of the coding region of the yeast *SLC1* gene used in the present invention, the stop codon being identified by "@", and a highly conserved consensus sequence among bacterial and yeast *sn*-2 acyltransferases being underlined;

Fig. 3 shows a strategy for constructing an *SLC1-1* plant transformation vector explained in the Experimental Details provided later, the salient features not being drawn to scale; and

Figs. 4 to 7, as well as Tables 1-20 below, show the results of tests explained in the Experimental Details provided later.

BEST MODES FOR CARRYING OUT THE INVENTION

The sequences of the *SLC1-1* gene [SEQ ID NO:1] and the *SLC1* allele [SEQ ID NO:3], and their derived peptide structures [SEQ ID NOS: 2 and 4], are as shown in Figs. 1 and 2, respectively.

The yeast *SLC1* gene (and related *SLC1-1* suppressor allele gene) have been characterized in two publications,

as follows (the disclosures of which are incorporated herein by reference):

1. Lester, R.L., Wells, G.B., Oxford, G. and Dickson, R.C. (1993) Mutant strains of *Saccharomyces cerevisiae* lacking sphingolipids synthesize novel inositol glycerolipids that mimic sphingolipid structures. *J. Biol. Chem.* 268: 845-856 - Reference 22; and

2. Nagiec, M.M., Wells, G.B., Lester, R.L., and Dickson, R.C. (1993) A suppressor gene that enables *Saccharomyces cerevisiae* to grow without making sphingolipids encodes a protein that resembles an *Escherichia coli* fatty acyltransferase. *J. Biol. Chem.* 268: 22156-22163 - Reference 25.

The DNA and amino acid sequences for the coding region of the *SLC1-1* gene are stored in GenBank/EMBL under accession No. L13282 (the stored sequence including a 5' untranslated region not disclosed in the present application).

The *SLC1* gene was originally cloned from a yeast mutant lacking the ability to make sphingolipids. The mutant allele of *SLC1* was shown to encode a protein which suppresses the genetic defect in sphingolipid long chain base biosynthesis. The gene sequence of *SLC1* is homologous to the *E. coli PLSC* gene, which has been claimed to encode *lyso*-phosphatidic acid acyltransferase (LPAT; an acyltransferase acylating the *sn*-2 position of *lyso*-phosphatidic acid (LPA) to give phosphatidic acid (PA)). The *SLC1* gene was able to complement the growth defect in JC201 (an *E. coli* strain mutated in *PLSC*). Based on the observation that *SLC* strains grown in the absence of long chain base make novel phosphatidylinositol derivatives (Lester et al., (1993) J. Biol. Chem. 268: 845-856.), one

possible conclusion by the authors was that the *SLC1* encodes a protein capable of acylating the *sn*-2 position of inositol-containing glycerolipids (i.e. perhaps an *lyso*-phosphatidyl-inositol acyltransferase, LPIT). Based on these findings, it was reported that *SLC1* encodes a yeast *sn*-2 acyltransferase. However, the authors of the paper (Dickson, Lester et al.), were unable to detect LPAT activity in the complemented *E. coli* JC201 mutant.

In the Nagiec et al. paper, the authors also reported the sequence of the gene for a suppressor allele designated *SLC1-1* in which nucleotide 131 has a T instead of an A, resulting in an amino acid change at position 44, from a glutamine to a leucine. The working hypothesis is that the *SLC1-1* suppressor allele encodes a variant acyltransferase with an altered substrate specificity, which enables it to use a very long-chain fatty acid (26:0) to acylate the *sn*-2 position of inositol-containing glycerolipids. The authors have not, to date, provided conclusive evidence of activity encoded by *SLC1-1* or *SLC1*.

Based on the interest of the inventors of the present invention in modifying the very long-chain fatty acid (VLCFA) content of Brassicaceae, the inventors obtained plasmid *p411 Δ B/C* containing the *SLC1-1* suppressor allele gene from Dr. Dickson at the University of Kentucky, Lexington, Kentucky, USA. The inventors also believed that expressing the foreign gene in a plant might lead to more information on the nature of what *SLC1-1* and *SLC1* encode. Work carried out by the inventors identified, for the first time, using the model oilseed *Arabidopsis thaliana*, transformants with increased seed oil content, and increased proportions of TAGs containing very long-chain fatty acids (VLCFAs = > C_{18}). In addition, there are increased proportions of VLCFAs at the *sn*-2 position of TAGs, and a concomitant decrease in the proportion of polyunsaturated fatty acids esterified at this position.

SLC1-1 transformants *of B. napus* cv. Hero and *B. carinata* (both high erucic acid cultivars) show increased oil content and increased erucic acid content/mg dry weight (DW) of seed. *SLC1-1* transformants of *B. napus* cv. Westar (Canola-quality cultivar) show increased proportions of oleic acid (18:1) and decreased proportions of polyunsaturated fatty acids (18:2 and 18:3).

The *SLC1*-1 and *SLC1* genes can be introduced into the genomes of oilseed plants and expressed using conventional genetic engineering techniques. For example, transformation could involve the use of *Agrobacterium Ti* plasmid-mediated transformation (e.g. *in planta*, vacuum infiltration, cotyledonary or hypocotyl petiole wound infection, or particle bombardment, etc). Constructs may be driven by constitutive or tissue-specific promoters, as will be apparent to persons skilled in the art.

Broad applicability of the invention to oilseed plants of various kinds is to be expected because oil synthesis follows the same or closely related biochemical pathways in all such plants (see References 29, 30, 37, 38, 39 and 40).

The present invention will be described in more detail with reference to the following experimental details, which provide specific illustration. It should be kept in mind, however, that the present invention is not limited to the details presented below.

EXPERIMENTAL DETAILS

CONSTRUCTION OF VECTORS FOR *SLC1-1* TRANSFORMATION

Following the cloning strategy illustrated in Fig. 3 of the accompanying drawings, two primers with 5′ BamHI restriction site extensions, OM087 (AGAGAGAGGGATCCATGAGTGTGATAGGTAGG) [SEQ ID NO: 5] and OM088 (GAGGAAGAAGGATCCGGGTCTATATACTACTCT) [SEQ ID NO:6], designed according to the 5′ and 3′ end sequences of the *SLC1* gene [SEQ ID NO:3], respectively, were used in a Polymerase Chain Reaction (PCR) with plasmid p411ΔB/C (obtained from

Dr. Dickson at the University of Kentucky, Lexington, Kentucky, USA), harboring the suppressor allele of the *SLC* gene (*SLC1-1*) as template, to generate the *SLC1-1* PCR fragment with a BamHI site at both ends. The (*SLC1-1*) PCR fragment, therefore, represents the suppressor allele of the *SLC1* gene with nucleotide T substituting for nucleotide A at position 131, resulting in an amino acid residue change from glutamine to leucine at residue 44. The fragment was digested with BamHI and ligated into the BamHI cloning site located between the tandem 35S promoter and NOS terminator in vector pBI524 (obtained from Dr. Raju S.S. Datla, NRC Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada, S7N OW9; published by Datla et al., 1993 - see Reference 9) to give vector *SLC1-1*-pBI-524. The orientation of *SLC1-1* in the vector *SLC1-1*-pBI-524 was verified by restriction digestion with BglII which cuts *SLC1-1* at nt 377 from the 5′ end and immediately downstream of the 35S promoter in vector pBI524. The translation initiation codon of *SLC1-1* is maintained, and hence the construct is a transcriptional fusion. The HindIII and EcoRI fragment containing a tandem 35S promoter, AMV enhancer, *SLC1-1* encoding sequence and NOS terminator was freed from *SLC1-1*-pBI-524, and cloned into the EcoRI-HindIII site of vector RD400 (also obtained from Dr. R. Datla; published by Datla et al., 1992 - see Reference 8). The final vector p*SLC1-1*/pRD400 (deposited on May 9, 1996 under the terms of the Budpest Treaty at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA; under deposit no. ATCC 97545) was introduced into *Agrobacterium tumefaciens* strain GV3101 (bearing helper plasmid pMP90; Koncz and Schell, 1986) by electroporation.

MOLECULAR BIOLOGICAL TECHNIQUES

Unless otherwise stated, all molecular biological techniques were carried out by methods generally prescribed by Ausubel et al., (1995).

PLANT GROWTH CONDITIONS

All *A. thaliana* control and transgenic plants were grown at the same time, in controlled growth chambers, under continuous fluorescent illumination (150-200 μE $m^{-2} \cdot sec^{-1}$) at 22°C, as described by Katavic et al., (1995). All other control and transgenic plants of the Brassicaceae (*B. napus, B. carinata*) were grown at the same time, in the P.B.I. Transgenic Plant Center greenhouse under natural light supplemented with high pressure sodium lamps (HPS lamps) with a 16 hour photoperiod (16 h light/8 h dark), at 22°C, and a relative humidity of 25-30%.

PLANT TRANSFORMATION

The *SLC1-1*/RD400 construct was tested in *A. thaliana* by *in planta* transformation techniques, and in both high and low erucic acid B. napus cultivars, and *B. carinata* (by co-cultivation transformation of cotyledonary petioles and hypocotyl explants with *A. tumefaciens* bearing the *SLC1-1* construct).

Testing the *SLC1-1* construct in *A. thaliana*

Wild type (WT) *A. thaliana* plants of ecotype Columbia were grown in soil. *In planta* transformation was performed by wound inoculation (Katavic et al. 1994) or vacuum infiltration (Bechtold et al. 1993) with overnight bacterial suspension of *A. tumefaciens* strain GV3101 bearing helper nopaline plasmid pMP90 (disarmed Ti plasmid with intact *vir* region acting *in trans*, gentamycin and kanamycin selection markers; Koncz and Schell (1986)) and binary vector p*SLC1-1* /pRD400.

After inoculation or infiltration, plants were grown to set seeds (T_1). Dry seeds (T_1) were harvested in bulk and screened on selective medium with 50 mg/L kanamycin. After two to three weeks on selective medium, seedlings were transferred to soil. Leaf DNA was isolated from kanamycin-resistant T_1 plants and analysed by PCR amplification of the *SLC1-1* fragment. Developing leaves from T_1 plants as well as T_2 mature seeds from *SLC1-1* transgenic lines were used for lipid and biochemical analyses. Developing leaves and mature seeds from untransformed wild type (WT) Columbia plants and pBI121 transgenic plants (binary vector pBI121, containing only kanamycin selection marker and GUS reporter gene; Jefferson et al., 1987) were used as controls in analyses of seed lipids. Based on these analyses, T_2 seeds of lines exhibiting changed acyl composition and/or lipid content were grown on selective medium (to eliminate homozygous WT segregants) and then transferred to soil to yield T_3 seed populations.

Testing the *SLC1-1* construct *in Brassica napus* and *Brassica carinata* :

Transformation experiments were also performed on *B. napus* cv. Westar (canola variety, low erucic acid), *B. napus* cvs. Hero, Reston and Argentine (all high erucic acid varieties) and *B. carinata* (breeding line C90-1163, a high erucic acid line) by co-cultivation of cotyledonary petioles and hypocotyl explants with *A. tumefaciens* bearing the *SLC1-1*/RD400 construct. Transformation methods according to Moloney et al.(1989) and DeBlock et al.(1989) were modified to optimize transformation conditions.

Modifications of the cotyledonary-petiole transformation method (Moloney et al., 1989) included the introduction of a 7-day explant-recovery period following co-cultivation, on MS medium with the hormone benzyladenine

(BA) and the antibiotic timentin, for elimination of *Agrobacterium*.

Modifications of the hypocotyl-explant transformation method (DeBlock et al.; 1989) included: (1) preculture of explants on agar-solidified MS medium with the hormones 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin (K); (2) co-cultivation of hypocotyl explants with *Agrobacterium* in petri dishes with the same medium as for preculture, on sterile filter paper; (3) following co-cultivation, a 7-day explant-recovery period on medium with hormones (2,4-D and K), and with timentin for *Agrobacterium* elimination, (4) regeneration of transgenic shoots on MS medium with the hormones benzyladenine (BA) and zeatin (Z), the ethylene inhibitor silver nitrate ($AgNO_3$), and antibiotics timentin (for *Agrobacterium* elimination) and kanamycin (for transformed-cell/shoot selection).

Green shoots were rooted and transferred to soil. Genomic DNA was isolated from developing leaves and PCR analyses and Southern analyses (Southern, 1975) were performed. Seeds (T_1) from transgenic plants were harvested and from each transgenic line, ten T_1 plants were grown in soil. Mature seeds (T_2) from these plants were harvested and subjected to lipid and biochemical analyses.

LIPID ANALYSES AND ACYLTRANSFERASE (LPAT) ASSAYS

Analyses of Leaf and Seed Lipids from *SLC1-1* and WT/pBI121 Transgenics and Untransformed WT plants

Lipids were isolated from mature seed and developing leaves as described previously (Taylor et al., 1992; Katavic et al, 1995) and analyzed by GC for total fatty acid content and fatty acid composition. Triacylglycerol species were analyzed by high-temperature GC as described by Katavic et al., 1995. Stereospecific analyses of TAGs

were performed on intact seed lipids (chiefly TAGs) as described by Taylor et al., 1994, 1995 a & b).

LPAT assays

For leaf assays, leaves at mid-expansion were chosen from control and *SLC1-1* transgenic plants, and leaf tissue sampled from several leaves with a cork-borer. For developing seed assays, in *A. thaliana* 25-30 silques were harvested at mid-seed development (15-18 d.p.a.) to give developing T_3 seed samples from both controls (untransformed WT and pBI121-transformed) and selected *SLC1-1* transgenics. *B. napus* and *B. carinata* T_2 embryos at the mid-cotyledonary stage of development were harvested from 3 siliques of control and selected *SLC1-1* transgenic plants. All plant material was frozen immediately in liquid nitrogen and stored at -70°C until homogenized. Homogenates of both plant leaf and developing seed tissues were prepared and LPAT assays conducted as described by Taylor et al., (1995b).

All protocols with respect to yeast strains were carried out as described by Ausubel et al., (1995, Unit 13.1 *Basic Techniques of Yeast Genetics*). Wild-type *S. cerevisiae* and *S. pombe* strains were cultured in YPD medium at 28°C at 270 r.p.m. overnight. At mid-log phase, cells were sampled, pelleted by centrifugation at 5,000 r.p.m. for 5 min, and resuspended in 100 mM Hepes-NaOH, pH 7.4. Cell lystes were prepared using acid-washed glass beads as described by Ausubel et al., 1995 (Unit 13.1, Section 13.13.4).

LPAT assays were conducted at pH 7.4, with shaking at 100 r.p.m., in a water bath at 30°C for 10-30 min. Assay mixtures (0.5 mL final volume) contained protein (10-200 µg, depending on the tissue/extract), 90 mM Hepes-NaOH, 0.5

mM ATP, 0.5 mM CoASH, 2 mM spermidine, 45 μM 18:1-LPA, and either 18 μM [1-^{14}C]-18:1-CoA, [1-^{14}C]-20:1-CoA, or [1-^{14}C]-22:1-CoA (each at a specific activity of 10 nCi/nmol) as the acyl donor. All other conditions for the measurement of LPAT activity are as detailed in Taylor et al (1995b).

^{1}H-NMR of Mature Seeds

^{1}H-NMR analyses for relative oil yield (Alexander et al., 1967; Rutar, 1989) were carried out on intact seeds of control and *SLC1-1*-transformed *B. napus* cv. Hero, and *B. carinata*, using a Bruker AM wide-bore spectrometer operating at 360 MHz. To reduce anisotropic line broadening, the seeds (35/sample) were rotated at 1 kHz in a zirconium rotor oriented 54.7° to the magnetic field (magic angle sample spinning, MASS).

RESULTS

Acyl-CoA Specificity of Yeast *(S. cereviseae; S. pombe)* *sn*-2 Acyltransferase (LPAT)

Yeast cell lysates from both *S. cereviseae* and *S. pombe* were assayed for relative *sn*-2 acyltransferase activity utilizing 18:1 LPA as an acyl acceptor and different radiolabeled acyl-CoAs. The acyl-CoA specificity of the yeast LPATs *in vitro* was quite broad, and the LPAT was capable of inserting both indigenous (16:0, 18:1) and non-indigenous (18:2, 18:3, 20:1, 22:1 and ricinoleoyl) acyl groups into the *sn*-2 position of 18:1 LPA, as shown in Table 1 below:

Table 1

Relative *S. cerevisiae* and *S. pombe* acyl-CoA: LPAT activities using 45 μM 18:1-LPA as acyl acceptor

^{14}C-Acyl-CoA supplied (18 μM)	LPAT Activity nmol/min/mg protein	LPAT Activity relative to 18:1-CoA (%)
S. cerevisiae		
18:1-CoA	3.75	100
18:2-CoA	3.54	94.5
18:1 Δ12-OH-CoA	1.90	50.7
20:1-CoA	1.92	51.3
22:1-CoA	0.33	8.9
S. pombe		
18:1-CoA	1.50	100
18:2-CoA	1.27	84.7
18:1 Δ12-OH-CoA	0.85	56.7
20:1-CoA	0.38	25.3
22:1-CoA	0.60	40.0

Because the yeast LPAT (*sn*-2 acyltransferase) has a relatively broad specificity, transformation of oilseeds rich in very long-chain fatty acids (*A. thaliana, B. napus*) with the yeast *SLC1-1* gene can be predicted to result in enriched VLCFA content, including the *sn*-2 position. In addition, yeast *SLC1* and *SLC1*-1 transformants can be predicted to be excellent hosts for transformation with hydroxylase genes from castor (*R. communis*) and *Lesquerella spp.* to produce seed oils enriched in hydroxy fatty acids. Alternatively, hydroxylase transformants may be sexually crossed with *SLC1*-1 or *SLC1* transformants.

A. thaliana SLC1-1 Transformant Seed Lipid Analyses:

Data from *Arabidopsis thaliana* transformation indicates that the gene has a dramatic effect on the total seed lipid content and *sn*-2 composition of TAGs. A large number of *SLC1-1* T_2 transgenic lines (21 of 48) showed significantly increased oil yields over untransformed controls, and pBI121 (without *SLC1-1* insert) controls, as shown in Table 2 below:

Table 2

Seed fatty acid contents of untransformed wild-type (u-WT) *A. thaliana*, pBI121(-*SLC1*-1) *A. thaliana* transformants (Controls) and selected T_2 transgenic lines of *A. thaliana* transformed with the yeast *SLC1*-1 gene. (Values are fatty acid content (μg)/50 seeds).

Line	16:0	18:0	18:1 c9	18:1c11	18:2	18:3	20:0	20:1	20:2	22:0	22:1	24:0 + 24:1	Total
u-WT Control	28.2	12.2	50.5	5.7	101.1	71.9	7.7	74.8	8.7	1.9	8.3	1.3	372.5
pBI121 Control	28.4	12.4	54.2	4.1	99.9	66.2	6.7	74.0	7.1	tr*	7.2	tr*	360.2
3	28.8	12.3	57.4	5.7	114.1	78.8	7.7	82.5	9.6	5.7	8.6	1.7	412.3
7	37.1	18.4	102.9	5.9	111.6	84.4	7.6	71.8	8.0	2.9	7.7	2.7	461.0
16	33.0	12.5	62.0	6.2	131.7	95.0	9.0	96.4	12.6	1.7	11.0	2.0	473.0
20	36.3	16.1	87.7	7.6	153.3	95.9	10.7	118.8	11.6	2.5	12.4	3.3	556.4
21	32.1	14.6	62.5	6.2	121.3	89.1	9.4	89.3	9.9	2.2	9.7	2.4	448.5

22	31.9	13.0	57.3	5.9	113.9	86.7	8.6	85.8	10.2	1.7	9.6	2.0	426.5
23	35.4	15.7	72.5	7.5	139.7	95.6	10.5	106.9	12.5	2.3	11.7	2.6	512.7
26	32.6	14.5	67.2	6.4	124.1	87.6	9.7	94.4	10.3	2.3	9.7	2.3	461.1
29	29.3	13.5	57.7	6.4	114.0	81.6	9.4	89.5	10.6	1.9	11.0	2.0	426.7
39	32.2	13.7	72.8	6.3	129.3	82.0	8.9	100.2	9.7	2.3	10.3	2.1	469.7
42	24.4	11.7	58.6	5.2	123.0	83.0	11.8	103.6	11.8	2.6	17.4	3.3	456.2
52	33.4	15.1	78.3	6.5	116.9	57.8	11.2	110.0	9.0	2.5	12.6	3.0	456.2
54	33.0	14.0	73.1	6.8	131.3	91.2	10.1	119.5	11.5	3.0	11.6	1.3	506.3

tr* = trace; < 0.2 wt%

In certain of these *SLC1*-1 T_2 lines, the proportion of VLCFA-containing TAGs (e.g. in Tables 3 and 4), and hence, seed content of total VLCFAs, especially eicosenoic acid and erucic acid, were dramatically increased (Table 5). In some cases, the overall proportions of VLCFAs were also increased (Table 6).

Those *SLC1*-1 transformed T_2 lines showing the most promising results in terms of increased oil content and increased proportions of VLCFA-containing TAGs, were selected and individual seeds planted to give T_3 progeny lines. Lipid analyses of TAGs from several independent *SLC1*-1 transgenic T_3 lines indicated that there was significantly increased total lipid content (reported as µg fatty acids/100 seeds; Table 7) which correlated with increased TAG content (nmol TAG/100 seeds; Table 8), compared to pBI121 Control T_3 transformants. In particular, the amounts of VLCFAs (µg/100seeds; Table 7) and levels of VLCFA-containing C_{58} and C_{60} TAGs (Table 8), were greatly enhanced in several *SLC1-1* transformants, over pBI121 control plants.

Stereospecific analyses of TAGs from selected independent T_3 *SLC1-1* transgenics contained increased proportions of VLCFAs (e.g. eicosenoic acid, 20:1) at the *sn*-2 position. This trend was consistent, regardless of whether the data was expressed as the proportion, among all *sn*-2 position fatty acids, which is represented by eicosenoic acid, or as the proportion of total eicosenoic acid in TAGs which is found at the *sn*-2 position (Table 9). Furthermore, in the *SLC1-1* transgenics, the increase in proportions of VLCFAs (e.g. eicosenoic acid) at the *sn*-2 position of TAGs was correlated with a concomitant decrease in the proportions of polyunsaturated fatty acids at this position, in comparison to pBI121 control plants (Fig. 4).

Table 3

TAG Species Accumulating in T_2 Seeds of Untransformed WT Control *A. thaliana*, and *SLC1-1* Transformant #42 (nmol /50 seeds ± SD)

Line	TAG C# →	C_{50}	C_{52}	C_{54}	C_{56}	C_{58}	C_{60}	Total
WT Con (n=5)	nmol ± SD	5.9 0.3	44.3 3.2	115.3 10.3	163.3 16.3	56.9 7.3	5.9 1.4	391.6 37.3
	mol % ± SD	1.5 0.1	11.3 0.4	29.5 0.7	41.7 0.4	14.5 0.8	1.5 0.3	100.0
	mol % C_{56}-C_{60}	57.7						
42 (n=2)	nmol ± SD	3.5 0.1	32.7 0.2	108.1 0.9	194.3 0.4	95.6 1.2	16.6 0.8	450.8 3.5
	mol % ± SD	0.8 0.01	7.2 0.01	24.0 0.004	43.1 0.3	21.2 0.1	3.7 0.2	100.0
	mol % C_{56}-C_{60}	68.0						

Table 4

TAG Species Accumulating in T_2 Seeds of Untransformed WT Control *A. thaliana*, and *SLC1-1* Transformant #16 (nmol /50 seeds ± SD)

WT Con (n=5)	nmol	5.9	44.3	115.3	163.3	56.9	5.9	391.6
	SD	0.3	3.2	10.3	16.3	7.3	1.4	37.3
	mol %	1.5	11.3	29.5	41.7	14.5	1.5	100.0
	SD	0.1	0.4	0.7	0.4	0.8	0.3	
	mol % C_{56}-C_{60}	57.7						
16 (n=2)	nmol	6.5	51.3	144.1	214.9	82.7	10.6	510.1
	SD	0.1	0.3	1.4	2.9	2.0	0.6	7.1
	mol %	1.3	10.1	28.3	42.1	16.2	2.1	100.0
	SD	0.04	0.1	0.1	0.02	0.2	0.1	
	mol % C_{56}-C_{60}	60.4						

Table 5

Eicosenoic (20:1), Erucic (22:1) and Total Very-Long Chain Fatty Acid (VLCFA) Content of T_2 Seed In Untransformed WT Control *A. thaliana* , pBI121 Controls and *SLC1-1* Transgenic Lines (µg / 50 seeds)

Line	20:1	22:1	Total VLCFAs
WT Con	74.8	8.3	102.8
SD (n=5)	6.4	0.7	10.1
pBI121 Con	73.8	7.0	96.7
SD (n=2)	2.3	0.3	3.4
16	96.4	11.0	132.6
20	118.8	12.4	159.2
23	106.9	11.7	146.4
42	103.6	17.4	150.3
52	110.0	12.6	148.2
54	119.5	11.6	156.8

Table 6

Proportions of Eicosenoic Acid (20:1), and Total VLCFAs in T_2 Seed of Untransformed WT Controls (u-WT), pBI121 Controls, and Selected *SLC1-1* Transgenic Lines of *A. thaliana* (wt % in 50-seed samples)

Line	20:1	All VLCFAs
u-WT Con	20.0	27.6
pBI121 Con	20.5	26.3
42	22.7	33.0
52	24.1	32.5
54	23.6	31.0

Table 7

Total Lipid Content (µg total FA /100 seeds) and VLCFA Content (µg /100 seeds) in Mature T_3 Seed of pBI121 Controls (pBI121 Con), and Selected *SLC1-1* Transgenic Lines of *A. thaliana* (µg / 100 seeds)

Line	Total Lipid Content	VLCFA Content
pBI121 Con a	483.5	119.7
pBI121 Con b	568.5	127.2
pBI121 Con c	519.7	125.1
pBI121 Con d	511.3	122.3
pBI121 Con Avg ± SE (n=4)	520.7 15.3	123.6 1.4
42-1	1137.9	315.5
42-4	851.7	218.6
42-5	984.6	268.0
23-8	1056.1	287.7
52-2	1109.2	307.5
52-5	870.0	253.3
52-6	1039.1	281.6
16-5	1955.3	227.0

Table 8

Total TAG Content and C_{58} and C_{60} TAG Content of Mature T_3 Seed of pBI121 Controls (pBI121 Con), and Selected *SLC1-1* Transgenic Lines of *A. thaliana* (nmol / 100-seed samples)

TAG C #→	C_{50}	C_{52}	C_{54}	C_{56}	C_{58}	C_{60}	Total
pBI121 Con	8.5	55.3	130.9	145.3	30.9	nd *	371.0
± SE (n=6)	0.4	2.6	7.8	9.0	2.7		21.6
16-5	12.4	88.2	214.7	251.6	70.5	5.6	642.9
23-8	17.7	130.8	333.6	409.0	106.8	8.0	1005.9
42-4	11.4	90.7	259.6	366.4	127.7	14.3	870.0
52-6	15.2	106.1	252.1	322.7	85.5	6.0	787.7

* nd = not detected

Table 9

Proportion of 20:1 at the *sn*-2 Position of TAGs (wt % ***sn*-2 20:1**) and Proportion of Total 20:1 Found at the *sn*-2 Position of TAGs (**wt % of total 20:1 at *sn*-2 position**) in Mature T_3 Seed of pBI121 Controls (pBI121 Con), and Selected *SLC1-1* Transgenic Lines of *A. thaliana* (wt % / 100-seed samples)

Line	wt % *sn*-2 20:1	wt % of Total 20:1 at *sn*-2 position *
pBI121 Con a	1.7	3.6
pBI121 Con b	0.6	1.1
pBI121 Con c	0.5	0.9
pBI121 Con d	1.6	3.0
16-5	4.2	16.3
42-1	5.1	8.5
42-4	7.9	12.8
42-5	5.3	8.7
23-8	7.5	12.0
52-2	6.2	10.0
52-5	5.8	9.7
52-6	7.5	12.0

* % of Total 20:1 in *sn*-2 position = (% in [*sn*-2 / [3 x % Total 20:1]] x 100)

B. napus and *B. carinata SLC1-1* Transformant Seed Lipid Analyses:

Several *B. napus* cv. Hero, cv. Reston, and *B. carinata SLC1-1* T_2 transformant seed lines exhibited increased oil content (Table 10) and increased erucic acid content, expressed as µg/ mg DW, or as µg/seed (Table 11). In *B. napus* cvs. Hero and Reston, seeds of several *SLC1-1* transgenic lines exhibited increased proportions of erucic acid (Table 12), compared to the corresponding levels in untransformed control plants. Single seed analyses from a selected average untransformed Hero plant (plant 4) and an *SLC1-1* transformant line with a promising high oil yield and high erucic acid phenotype (Line 8, plant 6) indicated a distribution of these traits suggestive of a seed population segregating in a typical Mendelian fashion for a single insert (Table 13). Some seeds of Hero Line 8 plant 6, exhibited probable homozygous WT (e.g. seed 8-6I) or homozygous *SLC1-1* (e.g. seeds 8-6K and 8-6H) phenotypes for all three traits (high oil yield, increased erucic acid content, increased proportions of erucic acid), while others displayed probable heterozygous WT/*SLC1-1* profiles with intermediate values for these three traits (e.g. seed 8-6B).

Table 10

Oil Yield (% Dry Weight) in T_2 Seeds of Untransformed Control (**Con**) and Selected *SLC1-1* Transgenic Lines of *B. napus* cvs. HERO and RESTON, and in *B. carinata* breeding line C90-1163 (± SE where applicable).

Line	Oil Yield (% DW)
***B. napus* cv HERO**	
Con	40.1 ± 1.7
5-1	46.7
5-4	48.7
7-3	45.3
7-6	46.4
7-9	44.9
8-4	45.9
8-6	50.9
8-7	44.9
8-10	45.1
***B. napus* cv RESTON**	
Con	33.4 ± 2.2
1-7	41.9
1-8	40.5
2-8	42.1
2-9	42.2
***Brassica carinata* line C90-1163**	
Con	35.9 ± 1.1
B. car 10-1-7	42.8
B. car 2-3-6	39.9

Table 11

Erucic Acid Content (expressed as μg / mg DW or μg / seed) in Mature T_2 Seeds of Untransformed Control (**Con**) and Selected *SLC1-1* Transgenic Lines of *B. napus* cv. HERO, and in *B. carinata* breeding line C90-1163 (± SE for Controls).

Line	22:1 (μg / mg DW)	22:1 (μg / seed)
***Brassica carinata* line C90-1163**		
Con	156.4 ± 5.6	--
10-1-7	180.4	--
***B. napus* cv HERO**		
Con	195.5 ± 11.7	596.7 ± 40.6
5-1	247.9	900.6
5-4	249.4	818.8
7-3	236.1	--
7-6	244.8	912
7-9	229.2	857.6
8-4	235.7	923.2
8-6	270.9	1020.3
8-7	238.5	888.3
8-10	232.7	900.4
3-1	--	--

-- not determined

Table 12

Proportions of Erucic Acid (expressed as wt %) in Mature T_2 Seeds of Untransformed Control (Con) and Selected *SLC1-1* Transgenic Lines of *B. napus* cvs. HERO and RESTON (± SE for Controls).

Line	wt % 22:1
B. napus **cv HERO**	
Con	48.6 ± 0.6
5-1	53.1
5-4	--
7-3	52.1
7-6	52.8
7-9	--
8-4	51.4
8-6	53.3
8-7	51.8
8-10	53.6
3-1	58.3
B. napus **cv RESTON**	
Con	34.7 ± 0.2
1-10	36.4
1-7	35.8
1-8	37.4
2-3	36.6
2-7	41.1

-- not determined

Table 13

Variation in Lipid Content (expressed as µg **total fatty acids /seed**) and Erucic Acid Content (expressed as µg **22:1** /seed or as **wt% 22:1**) in Mature T_2 Single Seeds of Untransformed Control plant 4 and *SLC1-1* Transgenic Line-8 plant 6 of *B. napus* cv. HERO (± SE for Averages, **AVG**).

Line /Seed	µg FAs / seed	µg 22:1 / seed	Wt % 22:1
AVG Con 4	1076.7 ± 61.5	507.1 ± 33.7	46.9 ± 0.8
AVG-8 6	1441.7 ± 67.3	735.4 ± 36.5	51.0 ± 0.6
8 6G	1324.8	710.8	54.1
8 6H	1704.3	877.1	52.5
8 6I	1175.4	557.3	47.4
8 6J	1206.8	629.4	52.2
8 6K	1694.7	911.1	53.8
8 6A	1351.6	658.6	48.7
8 6B	1304.5	670.6	51.4
8 6C	1221.1	639.1	52.3
8 6D	1449.0	714.3	49.3
8 6E	1678.2	844.6	50.3
8 6F	1748.0	876.8	50.2

There were measurable increases in the proportions of erucic acid and total VLCFAs at the *sn*-2 position in several transformant lines of Hero (Table 14). The effect of the yeast transgene on increasing the *sn*-2 erucic acid content in *B. napus* was somewhat less dramatic than its ability to change the *sn*-2 eicosenoic acid content in *A. thaliana* (c.f. Table 9). However, this is perhaps, not unexpected, based on the relative specificity of the *S. cerevisiae sn*-2 acyltransferase for eicosenoyl- *vs* erucoyl-CoA (c.f. Table 1).

Table 14

sn-2 Erucic Acid and VLCFA Content in Mature T_1 Seeds of Untransformed Control and Selected *SLC1-1* Transgenic Lines of *B. napus* cv. HERO.

Line/Seed	*sn*-2 22:1	*sn*-2 VLCFAs
Hero Control	1.5	3
Hero 8-6	2.8	4.6
Hero 8-6 G (single seed)	3.6	4.44
Hero 3-1	4.12	4.12 *
Hero 8-10	2.22	3.7

* Erucic acid (22:1) is the only *sn*-2 VLCFA detected.

Analyses of TAG species composition by GC, indicated that several *SLC1-1* transformant lines of Hero had increased proportions of C_{62} TAGs, and to a lesser extent, C_{64} and C_{66} TAGs (Table 15). The proportions of C_{62} - C_{66} TAGs containing 2 or more C_{22} fatty acids, was dramatically increased in Hero *SLC1-1* transgenics (Table 15), primarily at the expense of TAGs containing two (C_{56}) or three (C_{54}) C_{18} fatty acids (data not shown). A similar increase in the proportion of C_{62} TAGs was observed in some *B. napus* cv. Reston *SLC1-1* transgenic lines (Table 15).

Table 15

Proportions of C_{62}, C_{64} and C_{66} TAGs (mol %) in Mature T_2 Seeds of Untransformed Control (Con) and Selected *SLC1-1* Transgenic Lines of *B. napus* cvs. HERO and RESTON (±SE for Controls).

Line	C_{62}	C_{64}	C_{66}	Total C_{62}-C_{66}
Control	36.72 ± 1.42	1.32 ± 0.02	0.10 ± 0.01	38.14 ± 1.45
Hero 5-2	51.44	1.81	0.12	53.37
Hero 5-4	48.92	1.95	0.25	51.12
Hero 5-10	56.48	1.46	0.08	58.02
Hero 7-1	57.25	2.19	0.14	59.58
Hero 7-5	55.61	1.98	0.09	57.68
Hero 8-4	44.78	2.14	0.25	47.16
Hero 8-6	53.35	2.22	0.22	55.79
Reston				
Control	18.32	0.94	0.06	19.32
1-8	23.88	1.06	0.07	25.01
2-7	31.67	1.42	0.11	33.20

Analyses of typical control and *SLC1-1 B. napus* cv. Hero transgenics with respect to the seed-to-seed variation in proportions of C_{62} TAGs, indicated that the *SLC1-1* T_2 seed population was segregating, but that many of the single seeds had considerably higher proportions of C_{62} TAGs than any of the untransformed controls (Table 16).

Table 16

Single Seed Analyses for Proportions of C_{62} TAGs (mol %) in Mature T_2 Seeds of Untransformed Control (Con) and *SLC1-1* Transgenic Lines of *B. napus* cv. HERO (± SE for averages, AVG).

Line /Seed	C_{62} TAGs
Hero Con	
4d	38.54
4e	40.29
4b	36.88
4f	38.81
4g	30.05
4j	35.95
4h	42.84
4l	40.81
4k	43.28
Hero Con AVG	38.6 ± 1.35
Hero 8-6	
8-6 d	36.36
8-6a	47.63
8-6b	54.06
8-6c	54.81
8-6f	44.4
8-6g	56.27
8-6h	53.11
8-6l	42.19
8-6j	51.44
8-6k	58.4
Hero 8-6 AVG	51.35 ± 1.82

Estimates of oil yield increases in *SLC1-1* transgenic lines relative to contols, were directly correlated whether expressed on a "per mg dry weight" basis or on a "per seed" basis (Fig.5), as were estimates of relative oil content by a non-destructive ^{1}H-NMR method (Fig. 6). Indeed, the NMR results for increased oil yield were also positively correlated with increased seed weights in the *SLC1-1* transgenics (Fig. 7), and indicated that contributions to

increased seed dry weight were directly attributable to increased oil, with negligible contribution from seed water (absence of broad water resonance between the CH_2 OCO- and CHOCO- chemical shifts). Typical ^{1}H-NMR responses from 35-seed samples of control and "high oil" *SLC1-1* transgenic lines of *B. napus* cv. Hero and *B. carinata*, are depicted in Table 17.

Table 17

^{1}H-NMR Integral Response for Resonances Assigned to Liquidlike Oil (as described by Rutar; 1989) in Mature T_2 Seeds of Untransformed Controls and Selected *SLC1-1* Transgenic Lines of *B. napus* cv. HERO and *B. carinata* breeding line C90-1163. (35-seed samples; Responses relative to Control integration, set at 1.000)

Line	NMR Integral Response
***B. napus* cv HERO**	
Control	1.0000
Hero 5-1	1.5175
Hero 7-3	1.2721
Hero 7-6	1.3875
Hero 7-9	1.3245
Hero 8-4	1.5667
Hero 8-6	1.5297
Hero 8-7	1.4825
Hero 8-10	1.6302
***B. carinata* cv. C90-1163**	
Control	1.0000
B. car. 10-1-7	1.5977
B. car. 2-3-6	1.7548

Some *B. napus* cv. Westar (Canola) *SLC1-1* T_2 transformant seed lines showed increases in the relative proportion of oleic acid, and concomitant decreases in the relative proportions of polyunsaturated fatty acids (18:2 and 18:3) (Table 18). This is in contrast to the predicted effect as cited in the University of Kentucky patent application. Thus, the proportions of mono-unsaturated fatty acids can be increased in edible oils, by expression of *SLC1-1*. Furthermore, the proportions of saturated very long chain fatty acids in these Canola lines were significantly increased (Table 18).

Table 18

Oleic, Linoleic, Linolenic and Saturated VLCFA Compositions of Untransformed Control and Selected *SLC1-1* Transgenic Lines of *B. napus* cv. WESTAR (n=2 or 3)

Line	Oleic 18:1c9	Linoleic 18:2 c9,12	Linolenic 18:3 c9,12,15	Eicosanoic 20:0	Behenic 22:0	Lignoceric 24:0
***B. napus* cv WESTAR**						
Control	61.03	17.55	11.07	0.55	0.31	0.27
WS-13	70.03	14.80	3.41	0.76	0.49	0.56
WS-15	71.92	12.33	3.71	0.78	0.53	0.48
WS-16	71.06	12.29	3.87	0.97	0.59	0.56
WS- 15a	72.71	9.69	3.09	0.94	0.65	0.68

LPAT Analyses of Transformant Lines:

Samples of *B. napus* cv. Westar and *B. napus* cv. Argentine *SLC1*-1 T_1 transformant lines exhibited increased leaf 18:1-CoA:LPAT activities in rapidly-expanding leaf homogenate preparations compared to those from untransformed control plants (Table 19).

Developing seed LPAT analyses in untransformed control and *SLC1-1* transgenics of *B. napus* cv. Hero and *B. carinata* indicated that both 18:1-CoA:LPAT and 22:1-CoA:LPAT (Table 19) specific activities were dramatically increased in the *SLC1-1* transgenics.

Developing seed LPAT analyses of untransformed control and *SLC1-1* transgenics of *A. thaliana* indicated that 20:1-CoA:LPAT activity was increased in several *SLC1-1* transgenics (Table 19).

Thus, in this deposition we provide, for the first time, direct evidence that the yeast *SLC1-1* gene product encodes an enzyme which possesses *sn*-2 acyltransferase activity, and which can exhibit LPAT (EC 2.3.1.51) activity *in vitro*.

Table 19

Relative LPAT Activities in Homogenates Prepared from T_1 Leaf and T_2 or T_3 Developing Seed of Untransformed Controls and Selected *SLC1-1* Transgenic Lines of *B. napus* cvs. WESTAR, ARGENTINE and HERO, *B. carinata* cv. C90-1163, and *A. thaliana* cv. COLUMBIA. All assays conducted as described in experimental section.

Line	Tissue Assayed	LPAT Activity Assayed	DPM ^{14}C acyl-CoA incorporated into PA / µg pr
B. napus Westar	T_1 Leaves	18:1-CoA	
Control			307
WS 2-5			1008
WS 3-8			617
WS 6-7			1428
B. napus Arg.	T_1 Leaves	18:1-CoA	
Control			350
Arg 2-8			996
Arg 3-3			1557
B. napus Hero	T_2 Dev. Seeds	18:1-CoA	
Control			580
Hero 3-1			3470
Hero 7-6			2035
Hero 8-6			1370
B. car. C90-1163	T_2 Dev. Seeds	18:1-CoA	
Control			720
B. car 10-1-7			1125
B. napus Hero	T_2 Dev. Seeds	22:1-CoA	
Control			6.4
Hero 3-1			68.3
Hero 7-6			53.4
Hero 8-6			20.2
A. thaliana	T_3 Dev. Seeds	20:1-CoA	
WT u-Control			238
42-1			270
42-4			380
42-5			503

Genetic Analyses of *SLC1-1* Transformants:

PCR and Southern analyses data for the transgenic plant lines cited in this deposition are summarized in Table 20.

TABLE 20

Summary of PCR and Southern data
for *SLC1-1* T_2 transgenic plant lines (nd = not determined)

Oilseed	Transformant # (T_2 line)	PCR	Southern	Insert (Copy) #
A. thaliana	16	+	+	single
cv. COLUMBIA	20	+	+	single
	23	+	+	multiple
	42	+	+	multiple
	52	+	+	multiple
	54	+	+	multiple
B. napus	2	+	+	multiple
cv. WESTAR	3	+	+	multiple
	6	+	+	multiple
	13	nd	+	single
	15	nd	+	multiple
	16	nd	+	multiple
B. napus	2	+	+	multiple
cv. ARGENTINE	3	+	+	multiple
B. napus	5	+	+	single
cv. HERO	7	+	+	single
	8	+	+	single
	3	+	+	single
B. carinata	10	+	+	single
cv. C90-1163	2	+	+	multiple

To follow the segregation pattern in the T_2 generation of *A. thaliana SLC1-1* transformants, seeds from transgenic lines (e.g. lines 16, 20) which showed increases in oil content and amounts of long (C_{18}) and very long chain fatty acids (C_{20} and C_{22}) were sterilized and germinated on selective medium(50 mg/L kanamycin). Both lines showed the same 3:1 (kanamycin resistant:kanamycin sensitive) segregation pattern which indicates that the marker segregates as one Mendelian locus. Southern hybridization analyses (Southern, 1975) confirmed the presence of a single T-DNA insert per genome. In lines 23, 42, 52 and 54, Southern hybridization analyses suggest that all of the lines have more than one T-DNA insert per genome.

Northern hybridization analyses of seeds at mid-development isolated from siliques of *A. thaliana* lines 16, 20, 23, 42, 52 and 54 confirmed the expression of *SLC1-1* gene in all lines tested, with the highest level of expression in line 42.

Southern analysis of genomic DNA which was isolated from *B. napus* cv. Westar transgenic lines (2, 3, 6, 13, 15, 16) revealed that only line 13 had a single insert. Both *B. napus* cv. Argentine *SLC1-1* transgenic lines (2, 3) had multiple inserts. *B. napus* cv. Hero transgenic lines (3, 5, 7, 8) and *B. carinata* transgenic line 10, each had a single insert, while *B. carinata* line 2 had multiple T-DNA inserts per genome.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: National Research Council of Canada
(B) STREET: 1200 Montreal Road
(C) CITY: Ottawa
(D) STATE: Ontario
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): K1A 0R6

(A) NAME: Zou, Jitao
(B) STREET: #3E-1800 Main Street
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7H 2Z6

(A) NAME: Taylor, David C.
(B) STREET: 622 Wollaston Bay
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7J 4C3

(A) NAME: Katavic, Vesna
(B) STREET: 301 1121 C McKercher Drive
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7H 5B8

(A) NAME: MacKenzie, Samuel L.
(B) STREET: 17 Cambridge Crescent
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7H 3P9

(A) NAME: Keller, Wilfred A.
(B) STREET: 234 Emmeline Road
(C) CITY: Saskatoon
(D) STATE: Saskatchewan
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7J 5B6

(ii) TITLE OF INVENTION: MODIFICATION OF PLANT LIPIDS AND SEED OILS UTILIZING YEAST SLC GENES

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 947 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..909

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GTA CTG GCG CTT GCA GGC TGT GGC TTT TAC GGT GTA ATC GCC TCT ATC        96
Val Leu Ala Leu Ala Gly Cys Gly Phe Tyr Gly Val Ile Ala Ser Ile
            20                  25                  30

CTT TGC ACG TTA ATC GGT AAG CAA CAT TTG GCT CTG TGG ATT ACT GCG       144
Leu Cys Thr Leu Ile Gly Lys Gln His Leu Ala Leu Trp Ile Thr Ala
        35                  40                  45

CGT TGT TTT TAC CAT GTC ATG AAA TTG ATG CTT GGC CTT GAC GTC AAG       192
Arg Cys Phe Tyr His Val Met Lys Leu Met Leu Gly Leu Asp Val Lys
    50                  55                  60

GTC GTT GGC GAG GAG AAT TTG GCC AAG AAG CCA TAT ATT ATG ATT GCC       240
Val Val Gly Glu Glu Asn Leu Ala Lys Lys Pro Tyr Ile Met Ile Ala
65                  70                  75                  80

AAT CAC CAA TCC ACC TTG GAT ATC TTC ATG TTA GGT AGG ATT TTC CCC       288
Asn His Gln Ser Thr Leu Asp Ile Phe Met Leu Gly Arg Ile Phe Pro
                85                  90                  95

CCT GGT TGC ACA GTT ACT GCC AAG AAG TCT TTG AAA TAC GTC CCC TTT       336
Pro Gly Cys Thr Val Thr Ala Lys Lys Ser Leu Lys Tyr Val Pro Phe
            100                 105                 110

CTG GGT TGG TTC ATG GCT TTG AGT GGT ACA TAT TTC TTA GAC AGA TCT       384
Leu Gly Trp Phe Met Ala Leu Ser Gly Thr Tyr Phe Leu Asp Arg Ser
        115                 120                 125

AAA AGG CAA GAA GCC ATT GAC ACC TTG AAT AAA GGT TTA GAA AAT GTT       432
Lys Arg Gln Glu Ala Ile Asp Thr Leu Asn Lys Gly Leu Glu Asn Val
    130                 135                 140

AAG AAA AAC AAG CGT GCT CTA TGG GTT TTT CCT GAG GGT ACC AGG TCT       480
Lys Lys Asn Lys Arg Ala Leu Trp Val Phe Pro Glu Gly Thr Arg Ser
145                 150                 155                 160

TAC ACG AGT GAG CTG ACA ATG TTG CCT TTC AAG AAG GGT GCT TTC CAT       528
Tyr Thr Ser Glu Leu Thr Met Leu Pro Phe Lys Lys Gly Ala Phe His
                165                 170                 175

TTG GCA CAA CAG GGT AAG ATC CCC ATT GTT CCA GTG GTT GTT TCC AAT       576
Leu Ala Gln Gln Gly Lys Ile Pro Ile Val Pro Val Val Val Ser Asn
            180                 185                 190

ACC AGT ACT TTA GTA AGT CCT AAA TAT GGG GTC TTC AAC AGA GGC TGT       624
Thr Ser Thr Leu Val Ser Pro Lys Tyr Gly Val Phe Asn Arg Gly Cys
        195                 200                 205

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    210                 215                 220

GAC AAA ATT GGT GAA TTT GCT GAA AAA GTT AGA GAT CAA ATG GTT GAC       720
Asp Lys Ile Gly Glu Phe Ala Glu Lys Val Arg Asp Gln Met Val Asp
225                 230                 235                 240

ACT TTG AAG GAG ATT GGC TAC TCT CCC GCC ATC AAC GAT ACA ACC CTC       768
Thr Leu Lys Glu Ile Gly Tyr Ser Pro Ala Ile Asn Asp Thr Thr Leu
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                245                 250                 255

CCA CCA CAA GCT ATT GAG TAT GCC GCT CTT CAA CAT GAC AAG AAA GTG      816
Pro Pro Gln Ala Ile Glu Tyr Ala Ala Leu Gln His Asp Lys Lys Val
            260                 265                 270

AAC AAG AAA ATC AAG AAT GAG CCT GTG CCT TCT GTC AGC ATT AGC AAC      864
Asn Lys Lys Ile Lys Asn Glu Pro Val Pro Ser Val Ser Ile Ser Asn
        275                 280                 285
GAT GTC AAT ACC CAT AAC GAA GGT TCA TCT GTA AAA AAG ATG CAT          909
Asp Val Asn Thr His Asn Glu Gly Ser Ser Val Lys Lys Met His
    290                 295                 300

TAAGCCACCA CCACATTTTT AGAGTAGTAT ATAGACCC                            947
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 303 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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             20                  25                  30

Leu Cys Thr Leu Ile Gly Lys Gln His Leu Ala Leu Trp Ile Thr Ala
         35                  40                  45

Arg Cys Phe Tyr His Val Met Lys Leu Met Leu Gly Leu Asp Val Lys
     50                  55                  60

Val Val Gly Glu Glu Asn Leu Ala Lys Lys Pro Tyr Ile Met Ile Ala
 65                  70                  75                  80

Asn His Gln Ser Thr Leu Asp Ile Phe Met Leu Gly Arg Ile Phe Pro
                 85                  90                  95

Pro Gly Cys Thr Val Thr Ala Lys Lys Ser Leu Lys Tyr Val Pro Phe
            100                 105                 110

Leu Gly Trp Phe Met Ala Leu Ser Gly Thr Tyr Phe Leu Asp Arg Ser
        115                 120                 125

Lys Arg Gln Glu Ala Ile Asp Thr Leu Asn Lys Gly Leu Glu Asn Val
    130                 135                 140

Lys Lys Asn Lys Arg Ala Leu Trp Val Phe Pro Glu Gly Thr Arg Ser
145                 150                 155                 160

Tyr Thr Ser Glu Leu Thr Met Leu Pro Phe Lys Lys Gly Ala Phe His
                165                 170                 175

Leu Ala Gln Gln Gly Lys Ile Pro Ile Val Pro Val Val Val Ser Asn
            180                 185                 190

Thr Ser Thr Leu Val Ser Pro Lys Tyr Gly Val Phe Asn Arg Gly Cys
        195                 200                 205

Met Ile Val Arg Ile Leu Lys Pro Ile Ser Thr Glu Asn Leu Thr Lys
    210                 215                 220

Asp Lys Ile Gly Glu Phe Ala Glu Lys Val Arg Asp Gln Met Val Asp
225                 230                 235                 240

Thr Leu Lys Glu Ile Gly Tyr Ser Pro Ala Ile Asn Asp Thr Thr Leu
```

```
                245                 250                 255

Pro Pro Gln Ala Ile Glu Tyr Ala Ala Leu Gln His Asp Lys Lys Val
            260                 265                 270

Asn Lys Lys Ile Lys Asn Glu Pro Val Pro Ser Val Ser Ile Ser Asn
        275                 280                 285

Asp Val Asn Thr His Asn Glu Gly Ser Ser Val Lys Lys Met His
    290                 295                 300
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 947 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..909

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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GTA CTG GCG CTT GCA GGC TGT GGC TTT TAC GGT GTA ATC GCC TCT ATC        96
Val Leu Ala Leu Ala Gly Cys Gly Phe Tyr Gly Val Ile Ala Ser Ile
             20                  25                  30

CTT TGC ACG TTA ATC GGT AAG CAA CAT TTG GCT CAG TGG ATT ACT GCG       144
Leu Cys Thr Leu Ile Gly Lys Gln His Leu Ala Gln Trp Ile Thr Ala
         35                  40                  45

CGT TGT TTT TAC CAT GTC ATG AAA TTG ATG CTT GGC CTT GAC GTC AAG       192
Arg Cys Phe Tyr His Val Met Lys Leu Met Leu Gly Leu Asp Val Lys
     50                  55                  60

GTC GTT GGC GAG GAG AAT TTG GCC AAG AAG CCA TAT ATT ATG ATT GCC       240
Val Val Gly Glu Glu Asn Leu Ala Lys Lys Pro Tyr Ile Met Ile Ala
 65                  70                  75                  80

AAT CAC CAA TCC ACC TTG GAT ATC TTC ATG TTA GGT AGG ATT TTC CCC       288
Asn His Gln Ser Thr Leu Asp Ile Phe Met Leu Gly Arg Ile Phe Pro
                 85                  90                  95

CCT GGT TGC ACA GTT ACT GCC AAG AAG TCT TTG AAA TAC GTC CCC TTT       336
Pro Gly Cys Thr Val Thr Ala Lys Lys Ser Leu Lys Tyr Val Pro Phe
            100                 105                 110

CTG GGT TGG TTC ATG GCT TTG AGT GGT ACA TAT TTC TTA GAC AGA TCT       384
Leu Gly Trp Phe Met Ala Leu Ser Gly Thr Tyr Phe Leu Asp Arg Ser
        115                 120                 125

AAA AGG CAA GAA GCC ATT GAC ACC TTG AAT AAA GGT TTA GAA AAT GTT       432
Lys Arg Gln Glu Ala Ile Asp Thr Leu Asn Lys Gly Leu Glu Asn Val
    130                 135                 140

AAG AAA AAC AAG CGT GCT CTA TGG GTT TTT CCT GAG GGT ACC AGG TCT       480
Lys Lys Asn Lys Arg Ala Leu Trp Val Phe Pro Glu Gly Thr Arg Ser
145                 150                 155                 160

TAC ACG AGT GAG CTG ACA ATG TTG CCT TTC AAG AAG GGT GCT TTC CAT       528
Tyr Thr Ser Glu Leu Thr Met Leu Pro Phe Lys Lys Gly Ala Phe His
                165                 170                 175
```

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TTG GCA CAA CAG GGT AAG ATC CCC ATT GTT CCA GTG GTT GTT TCC AAT        576
Leu Ala Gln Gln Gly Lys Ile Pro Ile Val Pro Val Val Val Ser Asn
            180                 185                 190

ACC AGT ACT TTA GTA AGT CCT AAA TAT GGG GTC TTC AAC AGA GGC TGT        624
Thr Ser Thr Leu Val Ser Pro Lys Tyr Gly Val Phe Asn Arg Gly Cys
        195                 200                 205

ATG ATT GTT AGA ATT TTA AAA CCT ATT TCA ACC GAG AAC TTA ACA AAG        672
Met Ile Val Arg Ile Leu Lys Pro Ile Ser Thr Glu Asn Leu Thr Lys
    210                 215                 220

GAC AAA ATT GGT GAA TTT GCT GAA AAA GTT AGA GAT CAA ATG GTT GAC        720
Asp Lys Ile Gly Glu Phe Ala Glu Lys Val Arg Asp Gln Met Val Asp
225                 230                 235                 240

ACT TTG AAG GAG ATT GGC TAC TCT CCC GCC ATC AAC GAT ACA ACC CTC        768
Thr Leu Lys Glu Ile Gly Tyr Ser Pro Ala Ile Asn Asp Thr Thr Leu
                245                 250                 255

CCA CCA CAA GCT ATT GAG TAT GCC GCT CTT CAA CAT GAC AAG AAA GTG        816
Pro Pro Gln Ala Ile Glu Tyr Ala Ala Leu Gln His Asp Lys Lys Val
            260                 265                 270

AAC AAG AAA ATC AAG AAT GAG CCT GTG CCT TCT GTC AGC ATT AGC AAC        864
Asn Lys Lys Ile Lys Asn Glu Pro Val Pro Ser Val Ser Ile Ser Asn
        275                 280                 285

GAT GTC AAT ACC CAT AAC GAA GGT TCA TCT GTA AAA AAG ATG CAT            909
Asp Val Asn Thr His Asn Glu Gly Ser Ser Val Lys Lys Met His
    290                 295                 300

TAAGCCACCA CCACATTTTT AGAGTAGTAT ATAGACCC                              947
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 303 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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Met Ser Val Ile Gly Arg Phe Leu Tyr Tyr Leu Arg Ser Val Leu Val
 1               5                  10                  15

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            20                  25                  30

Leu Cys Thr Leu Ile Gly Lys Gln His Leu Ala Gln Trp Ile Thr Ala
        35                  40                  45

Arg Cys Phe Tyr His Val Met Lys Leu Met Leu Gly Leu Asp Val Lys
    50                  55                  60

Val Val Gly Glu Glu Asn Leu Ala Lys Lys Pro Tyr Ile Met Ile Ala
65                  70                  75                  80

Asn His Gln Ser Thr Leu Asp Ile Phe Met Leu Gly Arg Ile Phe Pro
                85                  90                  95

Pro Gly Cys Thr Val Thr Ala Lys Lys Ser Leu Lys Tyr Val Pro Phe
            100                 105                 110

Leu Gly Trp Phe Met Ala Leu Ser Gly Thr Tyr Phe Leu Asp Arg Ser
        115                 120                 125

Lys Arg Gln Glu Ala Ile Asp Thr Leu Asn Lys Gly Leu Glu Asn Val
    130                 135                 140
```

Lys Lys Asn Lys Arg Ala Leu Trp Val Phe Pro Glu Gly Thr Arg Ser
145 150 155 160

Tyr Thr Ser Glu Leu Thr Met Leu Pro Phe Lys Lys Gly Ala Phe His
165 170 175

Leu Ala Gln Gln Gly Lys Ile Pro Ile Val Pro Val Val Val Ser Asn
180 185 190

Thr Ser Thr Leu Val Ser Pro Lys Tyr Gly Val Phe Asn Arg Gly Cys
195 200 205

Met Ile Val Arg Ile Leu Lys Pro Ile Ser Thr Glu Asn Leu Thr Lys
210 215 220

Asp Lys Ile Gly Glu Phe Ala Glu Lys Val Arg Asp Gln Met Val Asp
225 230 235 240

Thr Leu Lys Glu Ile Gly Tyr Ser Pro Ala Ile Asn Asp Thr Thr Leu
245 250 255

Pro Pro Gln Ala Ile Glu Tyr Ala Ala Leu Gln His Asp Lys Lys Val
260 265 270

Asn Lys Lys Ile Lys Asn Glu Pro Val Pro Ser Val Ser Ile Ser Asn
275 280 285

Asp Val Asn Thr His Asn Glu Gly Ser Ser Val Lys Lys Met His
290 295 300

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AGAGAGAGGG ATCCATGAGT GTGATAGGTA GG 32

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GAGGAAGAAG GATCCGGGTC TATATACTAC TCT 33

References of Interest to the Present Invention

1. Alexander, D.E., Silvela, L.S., Collins, F.I. and Rodgers, R.C. (1967) Analysis of oil content of maize by wide-line NMR. *J. Am. Oil Chem. Soc.* 44: 555-558.

2. Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Stuhl, K. (1995) *Current Protocols in Molecular Biology*, Vols 1, 2 and 3.

3. Bechtold, N., Ellis, J., and Pelletier, G. (1993) *In planta Agrobacterium*-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *C R Acad Sci Paris, Sciences de la vie/Life sciences* 316: 1194-1199.

4. Brown, A.P., Coleman, J., Tommey, A.M., Watson, M.D., and Slabas, A.R. (1994) Isolation and characterization of a maize cDNA that complements a 1-acyl-*sn*-glycerol-3-phosphate acyltransferase mutant of *Escherichia coli* and encodes a protein which has similarities to other acyltransferases. *Plant Mol. Biol.* 26: 211-223.

5. Brown, A.P., Brough, C.L., Kroon, J.T.M. and Slabas, A.R. (1995) Identification of a cDNA that encodes a 1-acyl-sn-glycerol-3-phosphate acyltransferase from *Limnanthes douglasii*. *Plant Mol. Biol.* 29: 267-278.

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7. Coleman, J. (1992) Characterization of the *Escherichia coli* gene for 1-acyl-*sn*-glycerol-3-phosphate acyltransferase (*plsC*). *Mol. Gen. Genet.* 232: 295-303.

8. Datla, R., Hammerlindl, J.K., Panchuk, B., Pelcher, L.E. and Keller, W.A. (1992) Modified binary plant transformation vectors with the wild-type gene encoding NPTII. *Gene*, 211: 383-384.

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11. DeBlock, M., DeBrouwer, D., and Tenning, P. (1989) Transformation of *Brassica napus* and *Brassica oleracea* using *Agrobacterium tumefaciens* and the expression of the *bar* and *neo* genes in the transgenic plants. *Plant Physiol.* 91: 694-701.

12. Hitz, W.D., Mauvis, C.J., Ripp, K.G., Reiter, R.J., DeBonte, L. and Chen, Z. (1995) The use of cloned rapeseed genes for cytoplastic fatty acid desaturases and the plastid acyl-ACP thioesterases to alter relative levels of polyunsaturated and saturated fatty acids in rapeseed oil. *Proc. 9th Internat'nal Cambridge Rapeseed Congress UK*, pp. 470-472.

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Patents of Interest to the Current Invention

1. Calgene, Inc. (Patent Applicant); Inventors: Davies, H.M., Hawkins, D., Nelsen, J., Lassner, M.; PCT patent publication WO 95/27791. "Plant lysophosphatidic acid acyltransferases."

2. Calgene Inc. has been granted a US patent (WPI Accession No. 91-348069-48; *Biotech Patent News*, 6, 1992) governing the use of anti-sense technology in plant cells.

3. duPont de Nemours and Company (Patent Applicant; Inventors: Lightner, J.E., Okuley, J.J.; PCT patent publication WO 94/11516; Published European patent application EP 0668919. "Genes for microsomal delta-12 fatty acid desaturases and related enzymes from plants."

4. Nickerson Biocem. Ltd. (Patent Assignee); Inventors: Slabas A.R. and Brown, A.P.; PCT patent publication WO 94/13814; European patent publication EP 0673424. "DNA encoding 2-acyltransferases."

5. University of Kentucky Research Foundation (Patent Applicant); Authors: Dickson, R. et al.; unpublished pending US Patent Application Serial No. 434,039. "A technique for specifying the fatty acid at the *sn*-2 position of acylglycerol lipids."

Claims:

1. A transgenic oilseed plant characterized in that said plant has a genome incorporating an expressible yeast *SLC1-1* or *SLC1* gene.

2. A plant according to claim 1 characterized in that said plant exhibits improved seed oil yield and/or a different seed oil composition compared with a plant of the same type that does not contain said gene.

3. A plant according to claim 1 characterized in that said gene has the nucleotide sequence of SEQ ID NO:1.

4. A plant according to claim 1 characterized in that said gene has the nucleotide sequence of SEQ ID NO: 3.

5. A plant according to claim 1, claim 2, claim 3 or claim 4 characterized in that said plant produces non-edible oils.

6. A plant according to claim 1, claim 2, claim 3 or claim 4 characterized in that said plant produces edible oil.

7. A plant according to claim 1, claim 2, claim 3 or claim 4 characterized in that said plant is *Arabidopsis thaliana* modified to include said gene.

8. A plant according to claim 1, claim 2, claim 3 or claim 4 characterized in that said plant is a member of the *Brassicaceae* modified to include said gene.

9. A plant according to claim 1, claim 2, claim 3 or claim 4 characterized in that said plant is *Brassica napus* modified to include said gene.

10. A plant according to claim 1, claim 2, claim 3 or claim 4 characterized in that said plant is *Brassica carinata* modified to include said gene.

11. A plant according to claim 1, claim 2, claim 3 or claim 4 characterized in that said plant is selected from the group consisting of borage (*Borago* spp.), canola, castor (*Ricinus communis*), cocoa bean (*Theobroma cacao*), corn (*Zea mays*), cotton (*Gossypium* spp), *Crambe* spp., *Cuphea* spp., flax (*Linum* spp.), *Lesquerella* and *Limnanthes* spp., linola, nasturtium (*Tropaeolum* spp.), *Oenothera* spp., olive (*Olea* spp.), palm (*Elaeis* spp.), peanut (*Arachis* spp.), rapeseed, safflower (*Carthamus* spp.), soybean (*Glycine* and *Soja* spp.), sunflower (*Helianthus* spp.), tobacco (*Nicotiana* spp.) and *Vernonia* spp.

12. A seed of a transgenic oilseed plant characterized in that said plant has a genome incorporating an expressible yeast *SLC1-1* or *SLC1* gene.

13. A seed according to claim 11 characterized in that said gene has a nucleotide sequence of SEQ ID NO:1.

14. A seed according to claim 11 characterized in that said gene has a nucleotide sequence of SEQ ID NO: 3.

15. A seed according to claim 12, claim 13 or claim 14 characterized in that said seed produces non-edible oils.

16. A seed according to claim 12, claim 13 or claim 14 characterized in that said seed produces edible oil.

17. A seed according to claim 12, claim 13 or claim 14 characterized in that said seed is a seed of *Arabidopsis thaliana* modified to include said gene.

18. A seed according to claim 12, claim 13 or claim 14 characterized in that said seed is a seed of a member of the *Brassicaceae* modified to include said gene.

19. A seed according to claim 12, claim 13 or claim 14 characterized in that said seed is a seed of *Brassica napus* modified to include said gene.

20. A seed according to claim 12, claim 13 or claim 14 characterized in that said seed is a seed of *Brassica carinata* modified to include said gene.

21. A seed according to claim 12, claim 13 or claim 14 characterized in that said seed is a seed of a plant selected from the group consisting of borage (*Borago* spp.), castor (*Ricinus communis*), cocoa bean (*Theobroma cacao*), corn (*Zea mays*), cotton (*Gossypium* spp), *Crambe* spp., *Cuphea* spp., flax (*Linum* spp.), *Lesquerella* and *Limnanthes* spp., nasturtium (*Tropaeolum* spp.), *Oenothera* spp., olive (*Olea* spp.), palm (*Elaeis* spp.), peanut (*Arachis* spp.), safflower (*Carthamus* spp.), soybean (*Glycine* and *Soja* spp.), sunflower (*Helianthus* spp.), tobacco (*Nicotiana* spp.) and *Vernonia* spp.

22. Plasmid pSLC1-1/pRD400 (ATCC 97545).

23. *Agrobacterium tumefaciens* strain GV3101 characterized in that said strain has been modified to include a yeast SLC1-1 gene.

24. A method of producing a transgenic oilseed plant, characterized in that an expressible yeast *SLC1*-1 or *SLC1* gene is introduced into the genome of said plant.

25. A method according to claim 24 further characterized by down-regulating an indigenous gene that encodes *lyso*-

phosphatidic acid acyltransferase already present in the transgenic oilseed plant.

26. A method according to claim 24 further characterized by carrying out a second transformation to introduce a further gene for modifying the properties of the transformed plant.

27. A method according to claim 26 characterized in that said further gene is an hydroxylase gene from castor or *Lesquerella* spp.

28. A method according to claim 24 characterized in that said further comprises crossing said transgenic oilseed plant with a related oilseed transformant already containing an expressible foreign or indigenous transgene affecting oilseed composition, to produce oilseed plants yielding modified fatty acids.

29. A method of producing a transformed oilseed plant with improved tolerance to biotic and abiotic plant stresses, compared with an untransformed plant of the same type, characterized by introducing into the genome of said plant an expressible yeast *SLC1-1* gene or *SLC1 allele*.

30. A method of obtaining edible or inedible plant seed oil, characterized by growing an oilseed plant, harvesting seeds of said plant and extracting seed oil from said oilseeds, characterized in that said oilseed plant is a transgenic oilseed plant having a genome incorporating an expressible yeast *SLC1-1* or *SLC1* gene.

31. A method according to claim 28 characterized in that an indigenous gene that encodes *lyso*-phosphatidic acid acyltransferase already present in said transgenic oilseed plant is down-regulated.

SLC1-1 Gene

```
        1                   21                  41
        atgagtgtgataggtaggttcttgtattacttgaggtccgtgttggtcgtactggcgctt
1:      M  S  V  I  G  R  F  L  Y  Y  L  R  S  V  L  V  V  L  A  L
        61                  81                  101
        gcaggctgtggcttttacggtgtaatcgcctctatcctttgcacgttaatcggtaagcaa
21:     A  G  C  G  F  Y  G  V  I  A  S  I  L  C  T  L  I  G  K  Q
        121                 141                 161
        catttggctctgtggattactgcgcgttgtttttaccatgtcatgaaattgatgcttggc
41:     H  L  A  L  W  I  T  A  R  C  F  Y  H  V  M  K  L  M  L  G
        181                 201                 221
        cttgacgtcaaggtcgttggcgaggagaatttggccaagaagccatatattatgattgcc
61:     L  D  V  K  V  V  G  E  E  N  L  A  K  K  P  Y  I  M  I  A
        241                 261                 281
        aatcaccaatccaccttggatatcttcatgttaggtaggatttttccccccctggttgcaca
81:     N  H  Q  S  T  L  D  I  F  M  L  G  R  I  F  P  P  G  C  T
        301                 321                 341
        gttactgccaagaagtctttgaaatacgtccccttttctgggttggttcatggctttgagt
101:    V  T  A  K  K  S  L  K  Y  V  P  F  L  G  W  F  M  A  L  S
        361                 381                 401
        ggtacatatttcttagacagatctaaaaggcaagaagccattgacaccttgaataaaggt
121:    G  T  Y  F  L  D  R  S  K  R  Q  E  A  I  D  T  L  N  K  G
        421                 441                 461
        ttagaaaatgttaagaaaaacaagcgtgctctatgggtttttcctgagggtaccaggtct
141:    L  E  N  V  K  K  N  K  R  A  L  W  V  F  P  E  G  T  R  S
        481                 501                 521
        tacacgagtgagctgacaatgttgcctttcaagaagggtgctttccatttggcacaacag
161:    Y  T  S  E  L  T  M  L  P  F  K  K  G  A  F  H  L  A  Q  Q
        541                 561                 581
        ggtaagatccccattgttccagtggttgtttccaataccagtactttagtaagtcctaaa
181:    G  K  I  P  I  V  P  V  V  V  S  N  T  S  T  L  V  S  P  K
        601                 621                 641
        tatggggtcttcaacagaggctgtatgattgttagaatttaaaacctatttcaaccgag
201:    Y  G  V  F  N  R  G  C  M  I  V  R  I  L  K  P  I  S  T  E
        661                 681                 701
        aacttaacaaaggacaaaattggtgaatttgctgaaaaagttagagatcaaatggttgac
221:    N  L  T  K  D  K  I  G  E  F  A  E  K  V  R  D  Q  M  V  D
        721                 741                 761
        actttgaaggagattggctactctcccgccatcaacgatacaaccctcccaccacaagct
241:    T  L  K  E  I  G  Y  S  P  A  I  N  D  T  T  L  P  P  Q  A
        781                 801                 821
        attgagtatgccgctcttcaacatgacaagaaagtgaacaagaaaatcaagaatgagcct
261:    I  E  Y  A  A  L  Q  H  D  K  K  V  N  K  K  I  K  N  E  P
        841                 861                 881
        gtgccttctgtcagcattagcaacgatgtcaatacccataacgaaggttcatctgtaaaa
281:    V  P  S  V  S  I  S  N  D  V  N  T  H  N  E  G  S  S  V  K
        901                 921                 941
        aagatgcattaagccaccaccacatttttagagtagtatatagaccc
301:    K  M  H  @
```

FIG. 1

SUBSTITUTE SHEET

SLC1 Gene

```
     1                   21                  41
     atgagtgtgataggtaggttcttgtattacttgaggtccgtgttggtcgtactggcgctt
1:   M  S  V  I  G  R  F  L  Y  Y  L  R  S  V  L  V  V  L  A  L
     61                  81                  101
     gcaggctgtggcttttacggtgtaatcgcctctatcctttgcacgttaatcggtaagcaa
21:  A  G  C  G  F  Y  G  V  I  A  S  I  L  C  T  L  I  G  K  Q
     121                 141                 161
     catttggctcagtggattactgcgcgttgtttttaccatgtcatgaaattgatgcttggc
41:  H  L  A  Q  W  I  T  A  R  C  F  Y  H  V  M  K  L  M  L  G
     181                 201                 221
     cttgacgtcaaggtcgttggcgaggagaatttggccaagaagccatatattatgattgcc
61:  L  D  V  K  V  V  G  E  E  N  L  A  K  K  P  Y  I  M  I  A
     241                 261                 281
     aatcaccaatccaccttggatatcttcatgttaggtaggattttccccccctggttgcaca
81:  N  H  Q  S  T  L  D  I  F  M  L  G  R  I  F  P  P  G  C  T
     301                 321                 341
     gttactgccaagaagtctttgaaatacgtccccttttctgggttggttcatggctttgagt
101: V  T  A  K  K  S  L  K  Y  V  P  F  L  G  W  F  M  A  L  S
     361                 381                 401
     ggtacatatttcttagacagatctaaaaggcaagaagccattgacaccttgaataaaggt
121: G  T  Y  F  L  D  R  S  K  R  Q  E  A  I  D  T  L  N  K  G
     421                 441                 461
     ttagaaaatgttaagaaaaacaagcgtgctctatgggtttttcctgagggtaccaggtct
141: L  E  N  V  K  K  N  K  R  A  L  W  V  F  P  E  G  T  R  S
     481                 501                 521
     tacacgagtgagctgacaatgttgcctttcaagaagggtgctttccatttggcacaacag
161: Y  T  S  E  L  T  M  L  P  F  K  K  G  A  F  H  L  A  Q  Q
     541                 561                 581
     ggtaagatccccattgttccagtggttgtttccaataccagtactttagtaagtcctaaa
181: G  K  I  P  I  V  P  V  V  V  S  N  T  S  T  L  V  S  P  K
     601                 621                 641
     tatggggtcttcaacagaggctgtatgattgttagaattttaaaacctatttcaaccgag
201: Y  G  V  F  N  R  G  C  M  I  V  R  I  L  K  P  I  S  T  E
     661                 681                 701
     aacttaacaaaggacaaaattggtgaatttgctgaaaaagttagagatcaaatggttgac
221: N  L  T  K  D  K  I  G  E  F  A  E  K  V  R  D  Q  M  V  D
     721                 741                 761
     actttgaaggagattggctactctcccgccatcaacgatacaaccctcccaccacaagct
241: T  L  K  E  I  G  Y  S  P  A  I  N  D  T  T  L  P  P  Q  A
     781                 801                 821
     attgagtatgccgctcttcaacatgacaagaaagtgaacaagaaaatcaagaatgagcct
261: I  E  Y  A  A  L  Q  H  D  K  K  V  N  K  K  I  K  N  E  P
     841                 861                 881
     gtgccttctgtcagcattagcaacgatgtcaatacccataacgaaggttcatctgtaaaa
281: V  P  S  V  S  I  S  N  D  V  N  T  H  N  E  G  S  S  V  K
     901                 921                 941
     aagatgcattaagccaccaccacatttttagagtagtatatagaccc
301: K  M  H  @
```

FIG. 2

SUBSTITUTE SHEET

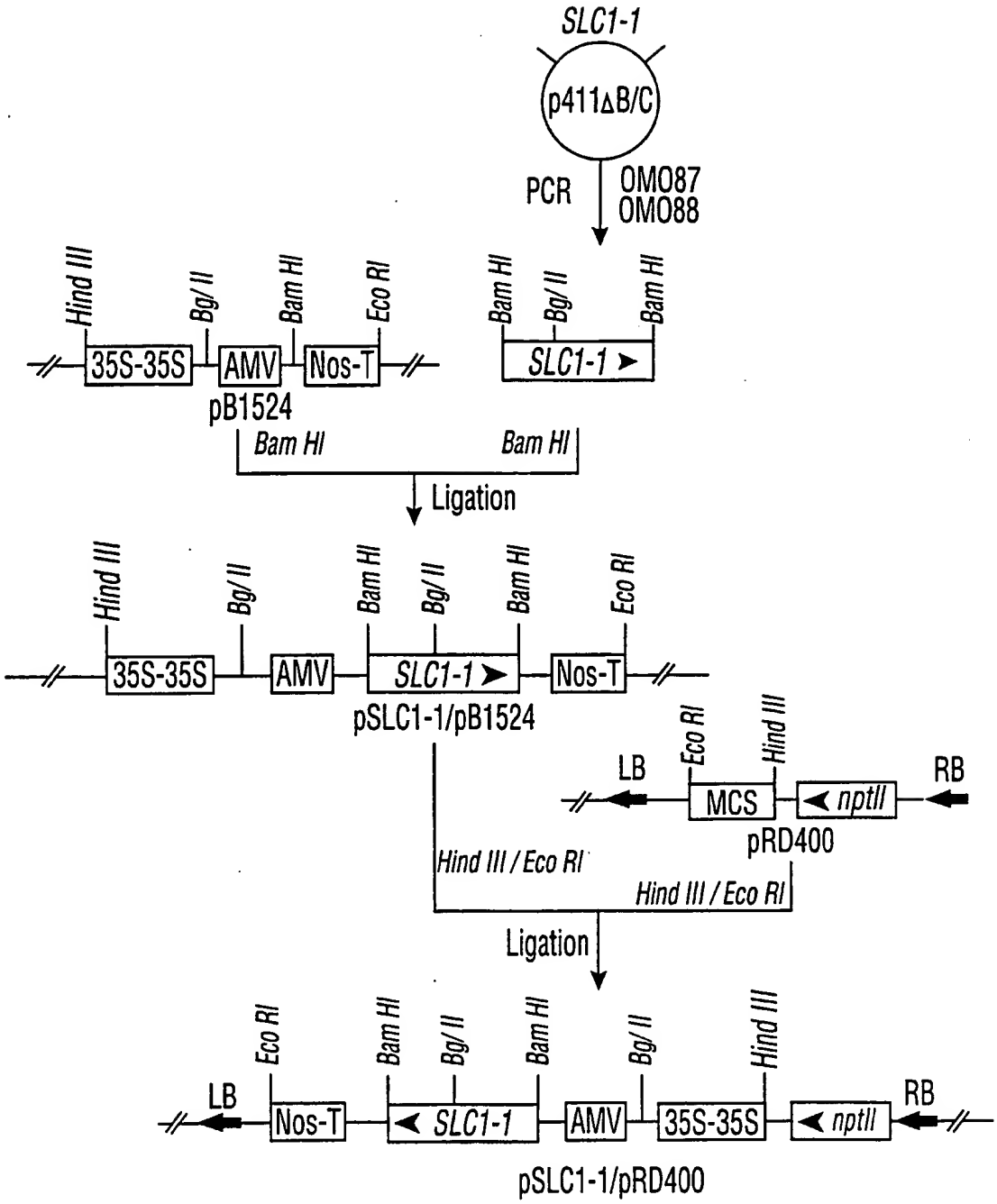

FIG.3 Cloning strategy for constructing SLC1-1 plant transformation vector (salient features not drawn to scale)

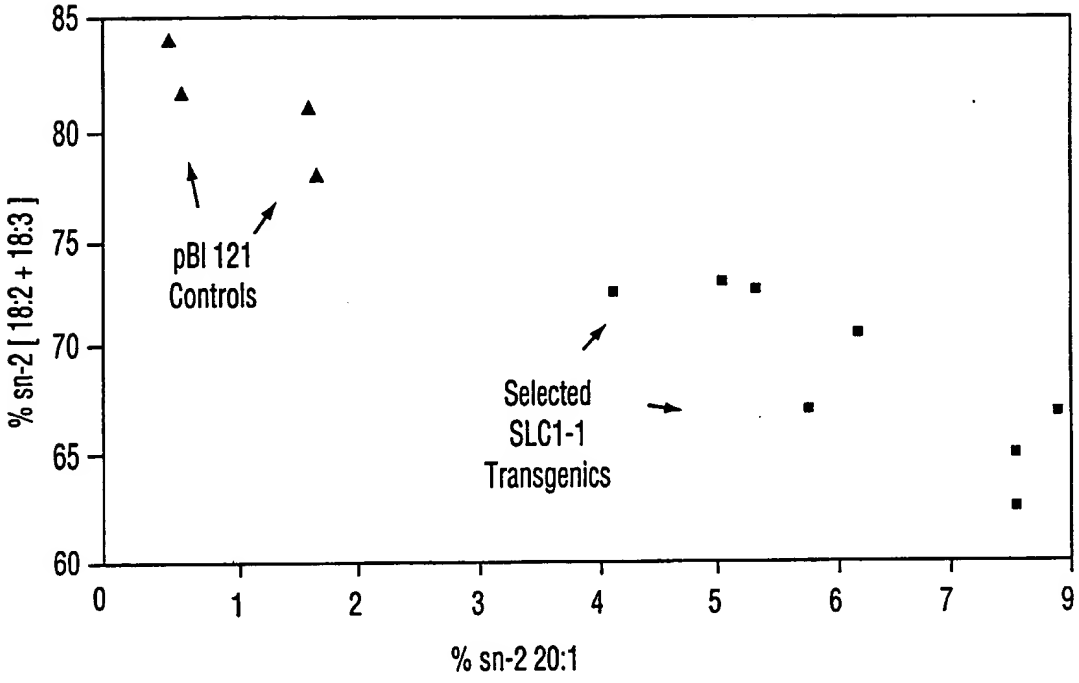

FIG. 4 Correlation between decrease in *sn-2* polyunsaturated fatty acids and increase in *sn-2* eicosenoic acid in A. *thaliana* Control and *SLC1-1* Transgenic T_3 Seeds.

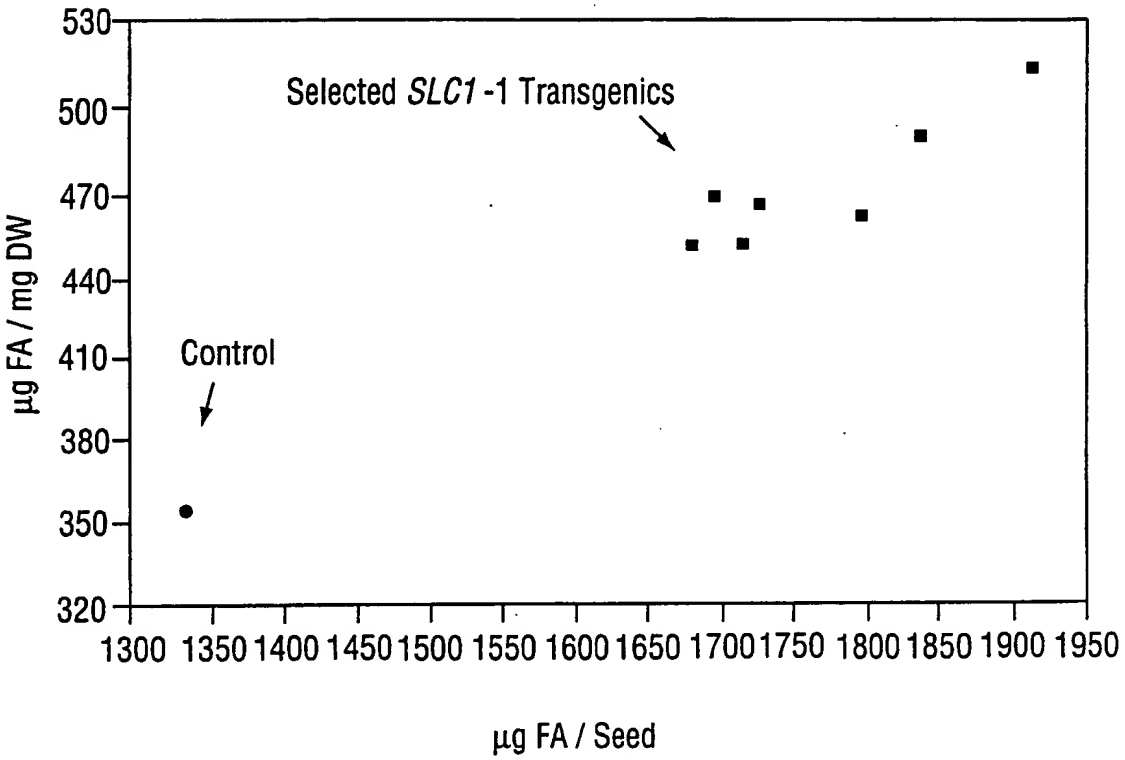

FIG. 5 Correlation of "Fatty Acid Content / Seed" and "Fatty Acid Content / mg Dry Weight" in Untransformed Control and Selected *SLC1-1* Transgenics of *B. napus* cv HERO (12 - Seed Samples)

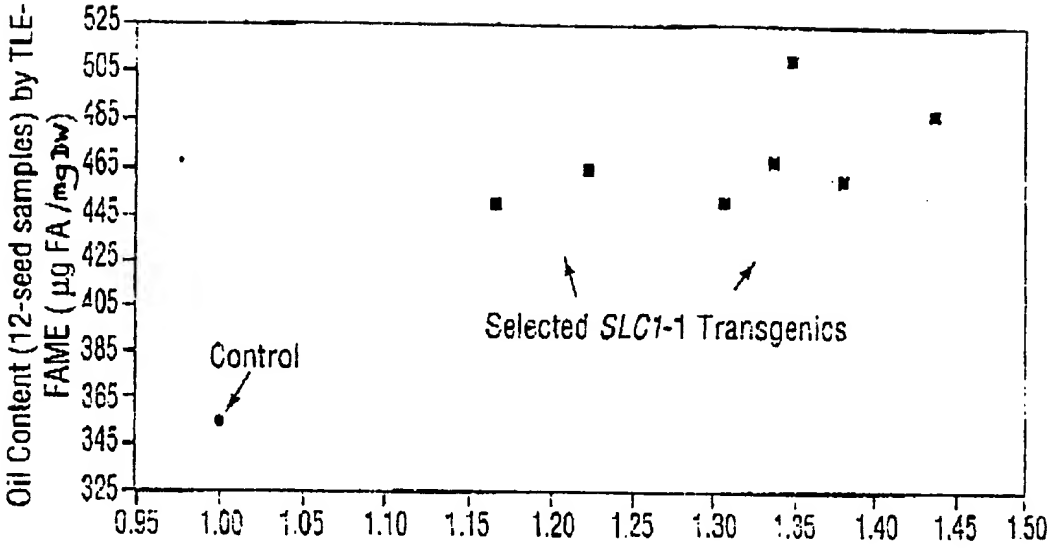

FIG. 6 Correlation Between Relative Oil Content Estimated by ^{1}H-NMR (non- destructive) Method *vs.* TLE-FAME (destructive) Method in Untransformed Control and Selected *SLC1-1* Transgenics of *B. napus* cv HERO

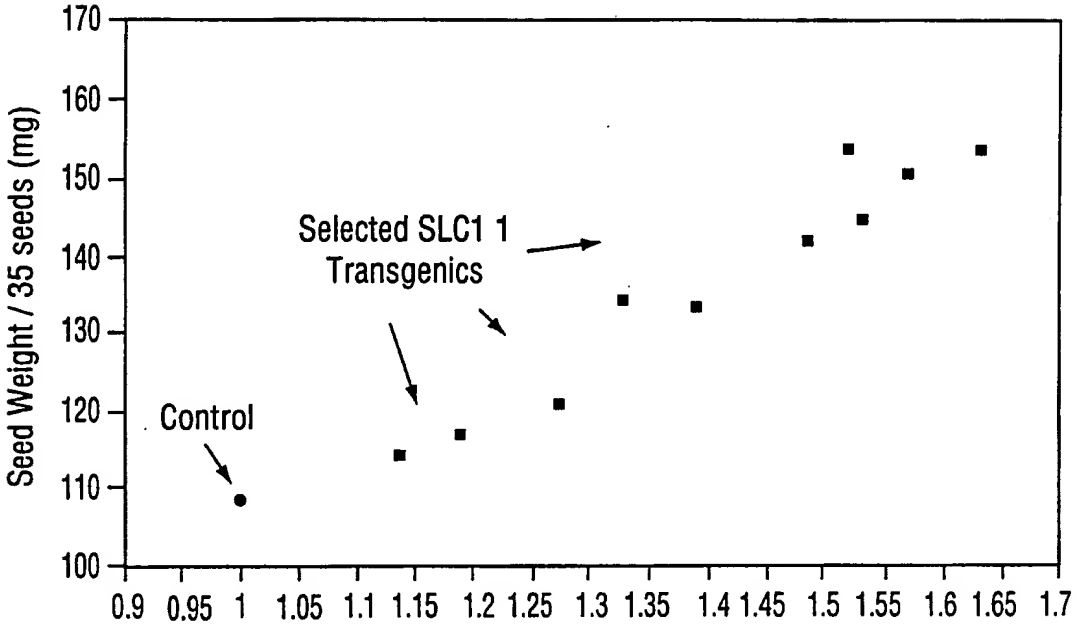

FIG.7 Correlation Between Seed Dry Weight and Oil Content (estimated by the non- destructive ^{1}H-NMR method) in Untransformed Control and Selected *SLC1-1* Transgenics of *B. napus* cv HERO

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/CA 96/00350

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 A01H5/00 A01H5/10 C12N1/21 C11B1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H C11B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,94 13814 (NICKERSON BIOCEM LIMITED) 23 June 1994 see the claims. ---	1
A	J. BIOL. CHEM., vol. 268, 1993, pages 22156-22163, XP002013481 M.M. NAGIEC ET AL.: "A suppressor gene that enables Saccharomyces cerevisiae to grow without making sphingolipids encodes a protein that resembles an Escherichia coli fatty acyltransferase" cited in the application see the abstract. --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
17 September 1996	02.10.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Yeats, S

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INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/CA 96/00350

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PLANT CELL ENVIR., vol. 17, 1994, pages 627-637, XP002013482 S. GIBSON ET AL.: "Use of transgenic plants and mutants to study the regulation and function of lipid composition" see the whole document. ---	1
P,A	WO,A,95 27791 (CALGENE INC.) 19 October 1995 see the claims. -----	1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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